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Progress in Biomedical Engineering

TOPICAL REVIEW

Polyaniline based polymers in tissue engineering applications: a review

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Abstract

A number of electrically conducting polymers, such as polyaniline (PANi), as well as functionalized aniline copolymers and composites, which are simultaneously biodegradable and conductive, have been applied for developing electrically conductive scaffolds for tissue engineering (TE) in recent years. The rationale behind these scaffolds is to induce 'electroactivity' in scaffolds, as many research works have shown that an intrinsic electrical activity leads to both increased regeneration rates and improved healing of damaged tissues. PANi is the conductive polymer of choice because it is economical and easy to process with a variety of methods. The resultant PANi based biomaterials have shown biocompatibility, conductivity, suitable processability, positive cellular response, as well as an intrinsic antibacterial effect in numerous research studies. The analysis of the literature has revealed that PANi based scaffolds have been investigated for TE applications including skin/wound healing, bone, cartilage, nerve/spinal cord, vascular, skeletal muscle repair and for the treatment of infertility. Although PANi based materials find widespread applications in other sectors, they are still far away from being commercially exploited as scaffolds for TE despite positive research results. This review aims to discuss and critically assess the current state of PANi based TE scaffolds for different applications. A future perspective for utilizing PANi based biomaterials for applications in TE is discussed, including recent considerations about potential cytotoxic effects.

1. Introduction

1.1. The tissue engineering (TE) approach and electrically conductive polymers

It is a well-known fact that there is a worldwide shortage of organ donors, and many patients die while waiting for an organ transplant as there is a huge mismatch between the number of required organs and patients requiring them. With increasing population and longer life span, this problem of donor scarcity will only further exacerbate. Thus an alternative is urgently required to prolong and to improve the lives of patients [1]. On the other hand, over the past decades a huge market for implants has developed, ranging from pace makers, vascular interventional devices to hip/knee implants. The implantable medical devices have also become more and more sophisticated and generally function well for a longer time period. However, there is still a high probability that they do not last for the lifetime of a patient, and therefore need to be repaired or replaced, often with huge detriment to the patient and a considerable burden to the healthcare system [2, 3]. A viable alternative to the current gold standard is regenerative medicine and TE as this technology theoretically has the ability to overcome the shortcomings of current approaches. TE as a biomedical field emerged more than 30 years ago although essential preliminary works preceded as early as 1971 [4]. This multidisciplinary field comprises a range of disciplines including materials science, cell and

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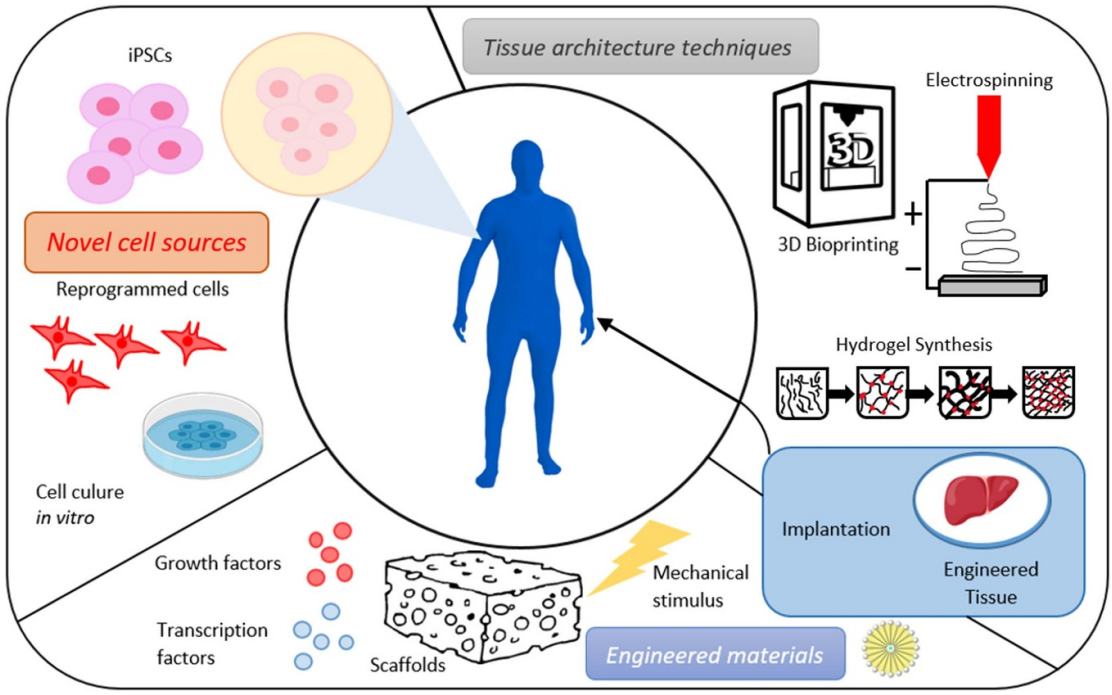

Figure 1. The TE paradigm.

molecular biology, bioengineering and clinical medicine, among others. There are many different strategies used in TE but most of them require importantly a 'TE scaffold' [4]. The scaffold is combined with cells native to the tissue to be regenerated as well as growth factors *in vitro* before it is implanted *in vivo*. Figure 1 shows the different aspects involved in the TE strategy, i.e. scaffolds, cells and processing technologies.

The scaffold should imitate the native tissue as closely as possible to provide the ideal environment for the residing cells to anchor and proliferate, ultimately leading to the complete regeneration of the damaged tissue or organ. The scaffolds are thus an integral part of the regeneration process. A scaffold is, generally speaking, a porous structure, which is bioresorbable in nature and provides a temporary structure for cells to proliferate and to reconstruct the native extracellular matrix (ECM) that has been damaged or diseased. Other important characteristics of TE scaffolds include mechanical stiffness matched to the stiffness of the surrounding tissue and the need to allow for the transportation of signalling factors and nutrients while at the same time enabling waste removal [3]. Furthermore, a scaffold should not be toxic or negatively affect the immune system, and needs to exhibit high reproducible quality. There is an enormous range of materials that have been and are being considered as TE scaffolds ranging from natural to synthetic polymers or blends of the two, as well as composites incorporating filler particles or fibres, hybrids, among others. In recent years, TE scaffolds have become increasingly complex incorporating filler particles in different size and nature as well as biomolecules and drug carriers [5].

In recent years, the usage of intrinsically conductive polymers has also become more and more widespread, incorporating polymers including polyaniline (PANi), polypyrrole (PPy), polythiophene as well as their derivates, such as poly(3,4-ethylenedioxythiophene) (PEDOT) [5]. The reason for using conductive polymers is that many tissues in the human body, including heart, bone, nerve, skeletal muscle and skin, are electrically excitable tissues, hence one can exploit this to achieve a better result. A search of the literature has shown that in recent years, the proposed applications areas for conductive PANi in the field of TE have increased away from its usage in 'traditionally electroactive tissues' such as cardiac and nerve tissue towards applications in bone [6–9], cartilage [10], skin [11–13], and others.

The reason for the increasing interest in PANi may be partly due to the fact that PANi is the most adaptive conductive polymer as it occurs in diverse structural forms (i.e. leucoemeraldine, emeraldine, pernigraniline), allowing relatively facile synthesis, and exhibiting environmental stability and excellent charge transport property due to the doping/dedoping process [7]. Another major advantage of PANi is its solubility in a few selected organic solvents, e.g. dimethylsulphoxide (DMSO), dimethylformamid (DMF) and HCOOH, etc. Recent developments in PANi synthesis have also led to PANi polymers which are soluble in water, thereby improving processibility largely. In fact, easier processing of PANi has enabled combining it

with other biodegradable and biocompatible polymers to fabricate simple 2D films to more hierarchical porous fibrous scaffolds and hydrogel systems. The biocompatibility of both pure PANi as well as it derivatives *in vitro* has been studied with a range of different cell types including dental pulp stem cells [11]; normal rat fibroblasts [14]; mouse embryo fibroblasts [15]; rat Schwann cells [16]; adipose stem cells [17]; mesenchymal stem cells [18]; cardiomyocytes [19]; rat endothelial progenitor cells [20]; pre-osteoblast cells (MC3T3-E1) [21] etc. However, to this date only a small number of *in vivo* studies has been performed so that the behaviour of PANi in terms of longevity of the electroconductive effect *in vivo*, biodegradability, biocompatibility, as well as unexpected side effects related to bioactivity are not yet studied well enough. Like other conductive polymers PANi is also non-biodegradable and in the last 20 years, numerous research studies have focused on blending PANi with other biodegradable and biocompatible natural or synthetic biopolymers to develop biodegradable PANi containing matrices for a variety of biomedical applications such as TE, biosensing, drug delivery and antimicrobial therapy. However, recently we also see more efforts in developing PANi incorporating doping moieties making it more conductive for long periods and in physiological medium. Similarly, more research has also been undertaken to develop PANi copolymers that are biodegradable. All these advancements have improved the biocompatibility of PANi, further cementing its candidature as a conductive matrix for TE applications.

1.2. Electrical stimuli (effects, applications, previous approaches)

Electrical stimuli combined with TE approaches are receiving increasing interest, which is based on a large body of evidence about the positive influence of electrical stimulation (EST) on regeneration processes. An early scientific work published in 1964 by Bassett *et al* [22] showed that the healing of bone can be positively influenced by means of applying an electrical current. In 2018, a study was published by Leppik *et al* [23] showing that EST in combination with TE was an effective means to treat a critical sized bone defect in a rat model. The study showed that the amount of new bone apposition as well as the quantity and density of newly formed blood vessels were significantly higher compared to the control group, whereas there was a significantly lower amount of fibrous tissue in the group subjected to EST. Another example for the healing effect of electricity is the technique used in clinical care to treat slow healing wounds using a high-voltage pulsed current simulator. The EST increases capillary density and perfusion, which leads to increased wound oxygenation, thus improving tissue granulation as well as fibroblast activity. The treatment is given for 1 h per day, 5–7 days a week and is used for a number of ailments, including chronic wounds, as well as pressure, diabetic or venous ulcers [24]. The application of an external electric field is obviously only possible when treating superficial wounds, whereas the inner organs cannot be treated in the same way as the electrodes need to be as close as possible. Moreover, as mentioned above, the treatment of chronic wounds with the use of an external electric field is time consuming and therefore expensive. Hence, the use of an internally applied indwelling device such as a conductive patch or scaffold would enable to employ the beneficial effect of targeted electricity. A conductive scaffold or patch can be fabricated either by incorporating conductive particles such as gold, graphene or carbon nanotubes (CNT) into a matrix or by using a conductive polymer such as PANi. A recent promising example of the incorporation of conductive nanoparticles (NPs) demonstrates that the repair of infarcted heart muscle in rats and minipigs could be achieved by means of an injectable and conductive scaffold, which consists of methacrylated elastin and gelatin with a CNT filler [25].

The ground breaking work of Nobel laureates Alan J. Heeger, Alan G. MacDiarmid and Hideki Shirakawa, who were awarded the Nobel prize in 2000 on conductive polymers, seems to have led to increasing interest in using CPs generally and for biomedical applications in particular [26]. More recently, CPs have been combined with electrically conductive nanofiller particles, such as carbon and graphene [15], which leads to increased conductivities.

Generally, polymeric materials are not conductive materials due to the fact that electrical charges have difficulty to transit through the material, however, some polymers, including PANi, are able to form a conductive path when an electric field is applied. The reason for this may be attributed to the molecular structure with conjugated chains with alternating single and double bonds. The structure of conducting PANi possesses a conjugated backbone (alternating single and double bonds), which gives rise to an extended π-network. The π-electrons in the structure, which are delocalized and polarizable, influence the electro-optical properties of PANi and other CPs. One of the original drawbacks for using PANi is that it can be difficult to process, for example using electrospinning, so it needs to be blended or co-polymerized [17, 21]. The conductivity of PANi can also be influenced by doping, which is usually done by an inorganic acid, such as hydrochloric or sulphuric acid. As a result of doping, a charge transfer reaction occurs, which in turn creates active sites (polarons), removing an electron from the valence band (p-doping) or adding an electron to the conduction band (n-doping).

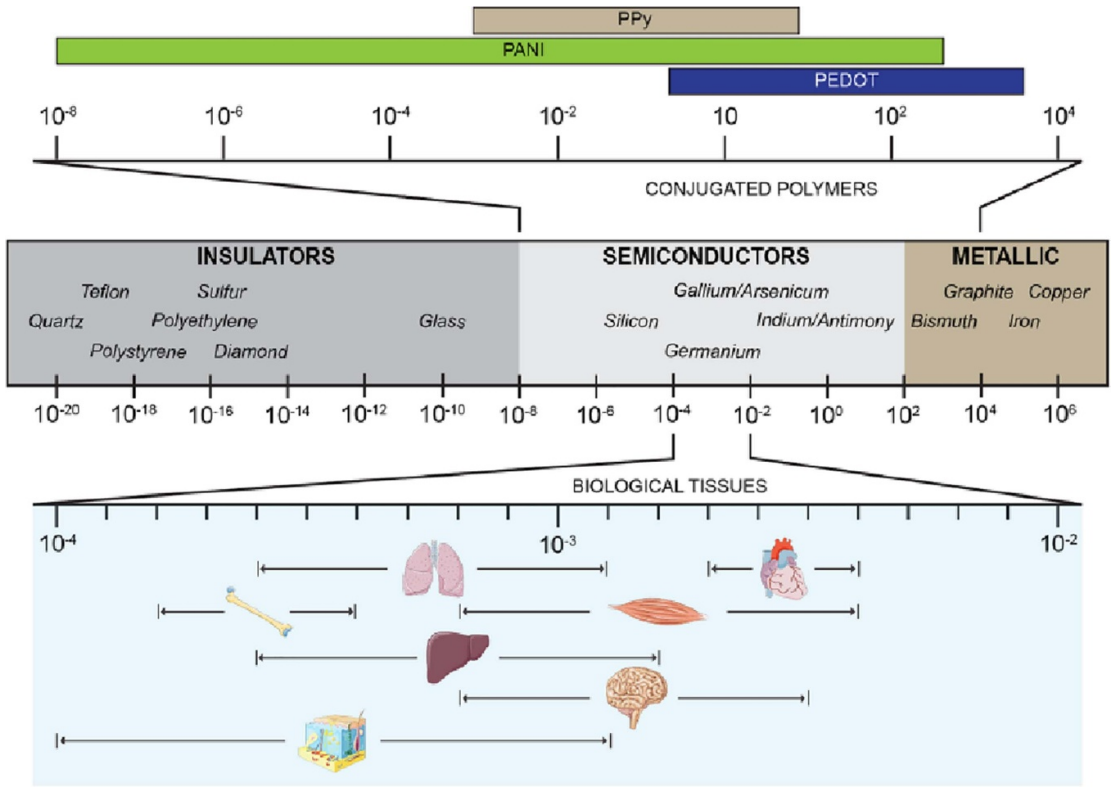

Figure 2. Schematic representation of the electrical conductivity ranges for three different CPs, i.e. PPy, PANi and PEDOT (top), material examples of insulators, semiconductors and metals (middle), and average conductivities for some biological tissues (bottom). Values are expressed in S cm^{-1}). Reproduced from [30]. CC BY 4.0.

Apart from the biochemical cues of a scaffold, it is also possible to influence cells using an electrical stimulus by means of activating many intracellular signalling pathways. Furthermore, the electrical stimulus influences the intracellular microenvironment, resulting in an increase in the migration, proliferation, and differentiation of cells. Therefore, the use of EST is of great interest in TE to maximize the efficacy of a TE scaffold [27]. The exact mechanism behind this effect, by which such systems influence cells, has not been fully understood yet and is the focus of current research. There have been some recent publications aiming at shedding light on this topic [27–30].

Figure 2 illustrates the electrical conductivity ranges of the most commonly used conductive polymers, i.e. PPy, PANi and PEDOT, together with the conductivity ranges of some of the biological tissues. PANI shows the greatest range of conductivity values, thus potentially making it a useful choice for different applications in TE.

A huge range of conductivities has been reported for PANi based TE scaffolds ranging from 10^{-4} to 10^{-1} S cm^{-1}, as shown in figure 2, be it simple polymer blends, co-polymer systems, or PANi based composites. Munawar and Schubert investigated a blend system, comprising poly(ethylene oxide) (PEO), polycaprolactone (PCL), poly(lactic acid) (PLA) and either PANi or PEDOT in different quantities to study the percolation threshold, which is defined as the critical volume fraction (ϕc) of a conductive filler inside a system, at which the values for electrical conductivity increase by orders of magnitude. Figure 3 illustrates the percolation phenomenon in electrospun electrically conductive nanofibers before and after annealing. The annealing process furthermore was shown to lead to a higher density of conductive channels within the nanofibrous meshes, which are responsible for increasing both intra- and interchain conductivities. By varying both the concentration of the different components of the blend fibres as well as the annealing temperatures, a large range of electrical conductivities up to more than 10^{-1} S cm^{-1} could be achieved, which means that the conductivity of the system is to some degree tailorable depending on the required conductivity for a given application [31, 32].

1.3. Motivation for this review

There has been an increasing interest in using PANi as a component in scaffold based TE, which is mirrored in the increasing number of research groups actively researching on the topic as well as the ever growing scientific literature. The number of research papers have increased enormously as can be seen clearly in the

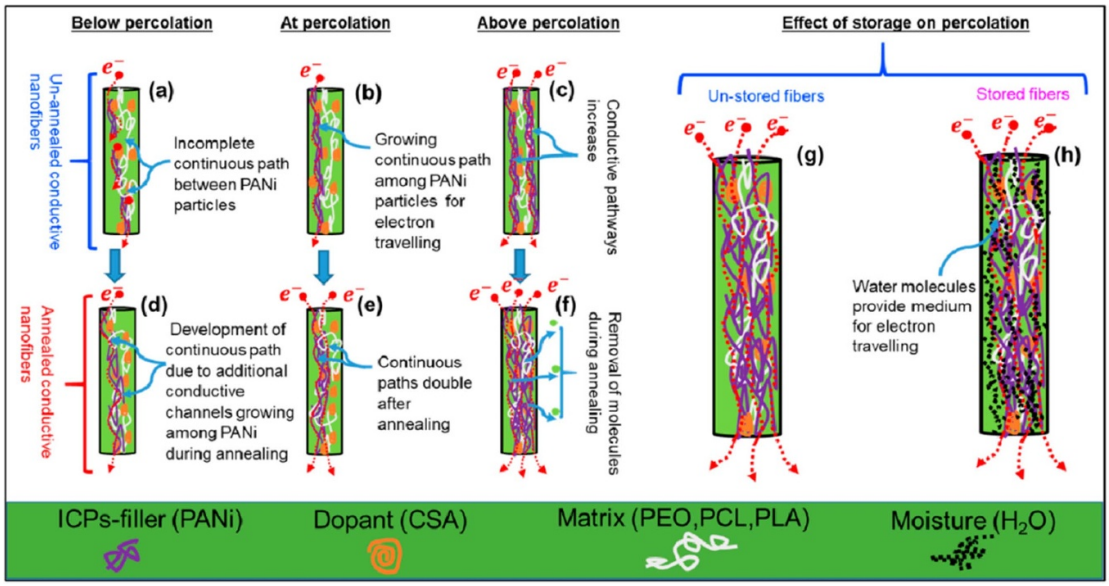

Figure 3. Schematic representation of the different stages of percolation within electrospun conductive nanofibers before and after annealing. 2(a)–(c) progressive elaboration of a continuous path of ICPs as a function of increasing filler concentration before annealing, 2(d)–(f) progressive elaboration of a continuous path of ICPs as a function of increasing filler concentration after annealing 2(g),(h) effect of storage for one day at room temperature; the storage resulted in water being absorbed into the nanofibrous structure, and the water molecules provided a medium between PANi particles for electron travel. Reproduced from [31]. CC BY 4.0.

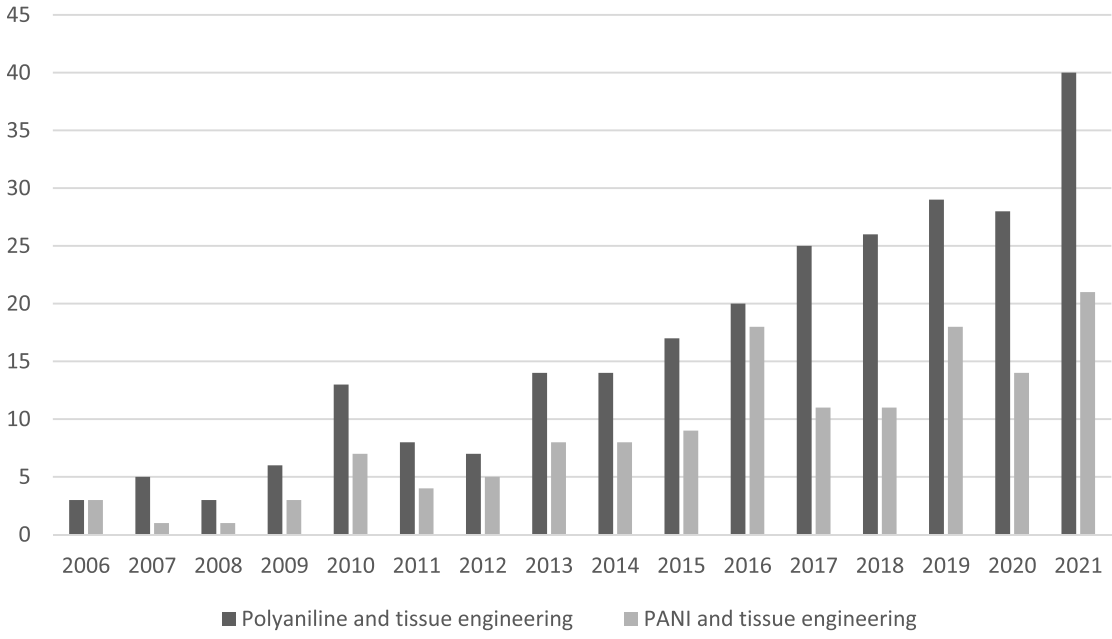

Figure 4. Graph showing the number of research publications with the keywords 'PANi' or 'polyaniline' and 'tissue engineering' using Scopus (last 15 years).

graph in figure 4 where the number of research publications from a literature search using the database Scopus with the keywords 'PANi' OR 'polyaniline' AND 'TE' is shown for the last 15 years.

There has also been a high number of review papers on the topic of PANi, PANi nanocomposites, PANI nanofibers or conductive polymers in general, some of which focus on a particular application, such as cardiac or nerve tissue regeneration or a particular processing technology, such as 3D printing, and some are focused on other applications outside biomaterials and TE [27–30, 33–53]. The large number of publications, including such a significant amount of review papers, indicates the very extensive amount of research efforts focusing on this topic worldwide. However, with this review paper, our motivation was to provide the readers with more wholesome information covering from basic TE concepts, i.e. the role that

scaffolds play in the success of the approach, to the importance of electrical stimuli. More specifically, we discussed the effect of electrical stimuli on excitable tissues and how providing such external electrical stimuli benefits tissue regeneration, focussing on information on the progress that has been made in this field to-date. Expanding on this, the historical perspective and previous applications of PANi have also been covered. The key properties that make PANi popular amongst the conductive polymers have also been discussed, supported with the core understanding of the conductive mechanism in PANi. Also, various processing methods, from choice of dopants to various fabrication approaches leading to final scaffold designs have been covered. Importantly, the review provides information on core properties and advantages of PANi such as aspects of biocompatibility, biodegradability and antibacterial activities. Finally, applications of PANi, particularly the co-polymers, blends and composites, in various TE applications are discussed. Thus, the review provides information covering topics that capture the progress and developments that have catapulted the popularity of PANi as a conductive matrix for TE applications.

2. Fundamentals on PANi

2.1. Short historical perspective, previous applications and key properties

The initial discovery of the CP PANi in the 19th century has been attributed to F. Ferdinand Runge, Carl Fritzsche, John Lightfoot and Henry Letheby. A comprehensive and interesting review of the history of discovery of PANi has been written in Seth C. Rasmussen, 2017 entitled 'The early history of PANi: discovery and origins', in which all the stages of discovery of PANi are detailed [26]. In 1960, the fact that some forms of PANi are indeed electroconductive was discovered and this work was recognised with Heeger, MacDiarmid and Shirakawa receiving the Nobel Prize in Chemistry entitled, 'for the discovery and development of electrically conductive polymers' in the year 2000. The three co-recipients of the award had started this collaborative work in the 1970s [26].

However, it must be mentioned that a number of predecessors before Heeger, MacDiarmid and Shirakawa had also carried out some fundamental work on CP. For instance, the first successful production of an organic polymer exhibiting significant conductivity has been attributed to Donald Weiss and his collaborators in Australia in 1963 who reported the synthesis of conducting PPy [26]. It was also Sapurina and Shishov, who first reported that PANi could be synthesized either by means of chemical or electrochemical oxidation of aniline monomers in acidic solution [54]. The charge carriers in the structure are formed during the oxidation process. PANi can either be synthesized by means of chemical or electrochemical oxidation of aniline monomers in acidic solution [54].

Owing to its electrical conductivity, PANi has been investigated intensively as a scaffold for TE applications. Once its antibacterial capability was discovered, the application of PANi also expanded into developing scaffolds that would support tissue regeneration as well as the fight against microbial infections. With more understanding and advancements in the processing and fabrication of PANi, its use as a scaffold continues, along with other biomedical applications such as development of antimicrobial therapies, drug delivery systems and as biosensors, such as a glucose sensor for diabetic patients [49]. In fact, the research interest in the field of biosensors has increased immensely in recent years as the technology greatly benefits from PANi doping-dedoping mechanism. We also see a huge expansion in the application of PANi in other areas beyond the biomedical field, especially in optical and electronic applications, such as solar cells, sensors, supercapacitors, electrochromic glasses, electroluminescent products, high tech clothing (wearable yarns), fuel cells, wastewater treatment, and energy storage [34, 39, 46, 51].

PANi is a versatile polymer, which can be synthesized in different forms and exhibits distinct properties depending on the oxidation level. These different forms are called pernigraniline (black and oxidized), emeraldine, either as insulator (emeraldine base (EB)) (blue) or conductive salt (emeraldine salt (ES)) (green and half-oxidized) and leucoemeraldine (pale and reduced). The chemical structures of each are shown in figure 5 [49]. The most stable configuration of PANi is emeraldine because every second nitrogen atom is oxidized, thus the polymer chain contains the same number of reduced and oxidized subunits [55]. The structure of PANi consists of reduced (x) and oxidized ($1 - x$) blocks ($0 \leqslant x \leqslant 1$), as shown in figure 5.

The electrical conductivity is influenced by the redox state, protonation degree, type and concentration of dopant and temperature, therefore the conductivity is tailorable to a given application. PANi can be synthesized using a range of different methods, including chemical or electrochemical oxidation [56] and self-assembling [57]. Khalid *et al* [58] developed a waterless synthesis of PANi, instead of using organic phase tetrahydrofuran (THF), as an alternative to the chemical polymerization method.

2.2. Electrical conductivity/mechanisms

As shown above, the structure of PANi consists of repeating units of the monomer aniline, which are connected to form a long backbone, as shown in figure 5. The presence of the N-atom between the phenyl

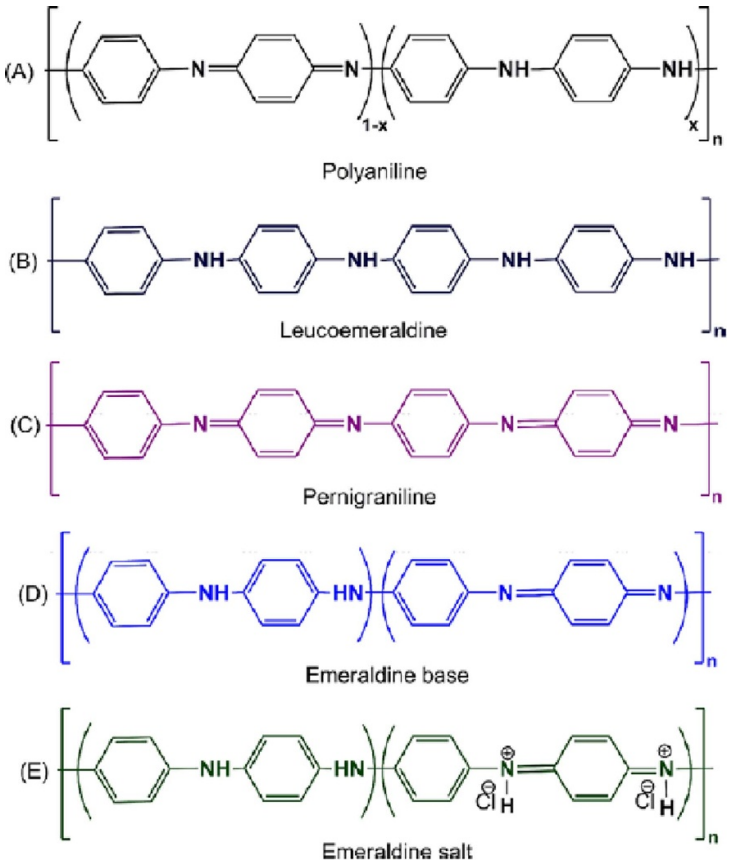

Figure 5. Schematic showing the different forms of PANI, (A) polyaniline, (B) leucoemeradine, (C) pernigraniline, (D) emeraldine base (EB), (E) emeraldine salt (ES). Reprinted with permission from [49]. Copyright (2019) American Chemical Society.

rings enables the formation of different oxidation states, which affect the physicochemical and mechanical properties of PANi significantly. The backbone of PANi consists of alternating single and double bonds along the monomer units. Already in the 1980's it had been postulated that PANi can be rendered conductive by protonation of EB leading to the conductive form ES through acid base chemistry. They proposed the usage of functionalized protonic acids in order to make PANi processable in its conducting form. The process involves a structural change with one unpaired spin per repeat unit. The protonation of EB results in the formation of a spinless charged structure (ESI) (bipolaron), which further rearranges to a charged radical open-shell structure ESI' (polaron), which finally splits into two ESII units (polaron) [54, 55]. The delocalized bonding electrons produce the electrical pathway for mobile charge carriers, which can be introduced by means of doping [54]. Doping is an effective way to improve the conductivity greatly, however the conductivity can be further enhanced by addition of inherently conductive materials, one example is the addition of CNTs, which were shown to act as a conductive bridge between the conductive domains inside the PANi structure, thus boosting the conductivity of PANi NPs [59].

The electrical conductivity in CPs occurs by means of transfer of electron from π type bonds to nearby simple σ bonds, because of repulsion between equal charges. The macromolecular polymer chains contain heteroatoms (i.e. N, S or O), and the electron transfer takes place from π-type bonds to the p electrons of heteroatoms, and subsequently moving to σ single bonds, which further leads to the movement of π electrons from nearby double bonds by the above mentioned electrostatic repulsion effect [29]. The conductivity of CPs substantially increases by oxidative (p-doping) and reductive doping (n-doping), i.e. the dopant type and concentration influence the electrical conductivity strongly.

The movement of charges in PANi and other conductive polymers can be accomplished through 'donor-acceptor' reversible chemical reactions, following equation (1):

$$Ox + ne^{-} \rightleftarrows \text{Red.} \tag{1}$$

The conduction of charges (or electrical conductivity) in a system is always proportional to the product of the density of charge carriers (n), the charge carried by each carrier (e) and the mobility of each carrier [60].

The Hall effect, which describes the production of a voltage difference across an electrical conductor, can be used to estimate the intrinsic carrier concentration, its mobility and the type of carrier for a semiconductor. For positively charged carriers (p-type semiconductors) the Hall voltage is positive and vice versa (i.e. negative Hall voltage indicates n-type). As determined in experimental studies, the majority of charge carriers in PANi are empty spaces, which suggests that PANi is a p-type semiconductor [60]. The semi-conducting properties result from the delocalized π bonds in the system. Electrical properties of CPs are determined by the band gap value. As the band gap energy decreases, transition becomes easier, resulting in a high conductivity. Different mechanisms of electrical conduction in PANi have been proposed [28, 29, 61, 62].

The band gap theory model proposes that the electrical conductivity of a CPs can be changed by either removing electrons from the valence band or by the use of an oxidizing agent, which creates a positive charge, or by donating an electron to the empty conduction band using a reducing agent. There are several techniques available for doping a CP including (a) electrochemical doping, (b) gaseous doping, (c) solution doping, (d) self-doping, and (e) radiation induced doping. It is also possible to dope a CP by irradiating the polymer using neutrons [62].

Almasi *et al* studied the band gap energy of PANi (EB) and PANI/multi-walled carbon nanotubes (MWCNT) composites and found that with increasing content of nanotubes the band gap value decreased significantly while the electrical conductivity increased tenfold [63].

2.3. Processing methods

Processing is a very important step as it entails the final fabrication approach of the material of interest into a shape that supports the target application. In the context of PANi, many parameters impact its processing and final outcome. To begin with, the very approach used for its synthesis can have a significant influenceon its further processing. Most commonly, PANi is synthesized via chemical oxidative polymerization of aniline monomer in aqueous media with the help of an oxidizing agent. However, there are a number of other chemical methods that can be used for the synthesis of PANi, which include solution, emulsion, dispersion, metathesis, interfacial and self-assembling polymerization [64].

The different synthesis methods used and the choice of starting precursors can further impact the selection of dopant and all of these combined can impact the properties of the final PANi product, thus affecting greatly its processing. For instance dopants are known to affect the conductivity and solubility of PANi in solvents. In fact, the use of secondary dopants has also been reported to enhance the conductivity of PANi by several orders of magnitudes when compared to only being treated with a primary dopant [33]. Dopants have also been shown to affect the surface roughness of scaffolds thereby impacting interactions with cells [34]. In general, the processing of PANi has concentrated on using blends with either natural or synthetic polymers (or combinations of both), co-polymers of PANi with other biocompatible and bioresorbable polymers or composites, which also incorporate other polymers as well as a nanofiller phase. The properties of conductive scaffolds in terms of microstructure, physicochemical, mechanical and degradation properties as well as the electrical conductivity obviously vary depending on the type of tissue in question as the scaffold needs to match the tissue characteristics as closely as possible.

Manifold processing methods for conductive TE scaffolds based on PANi are available including electrospinning, 3D printing, thermally induced phase separation, and direct mixing, as well as less commonly chemical oxidative polymerization. A green approach by the Michael addition reaction has also been reported [65]. There are comprehensive publications available in the open literature, and for TE the fabrication methods may be broadly subdivided into three different groups, i.e. electrospinning, hydrogel systems and 3D printing, which are briefly outlined in the following sections.

2.3.1. Electrospinning

First discovered in the late 19th/early 20th century, the electrospinning method slowly started to gain in popularity in the last few decades after Sir Geoffrey Ingram Taylor started to develop the fundamental theory of the process by mathematically modelling the shape of the (Taylor) cone formed by the fluid droplet at the tip of the syringe in the presence of an electric field in the 1960s. The process requires a high voltage source, a syringe pump with a syringe attached and a collector [66]. The process is very versatile, and particularly interesting for TE, as micrometric or nanosized fibres are generated which resemble the native ECM closely. PANi, in particular in its form of ES, is almost insoluble in most organic solvents. On the other hand further research has shown that PANi and PANi based materials can be dissolved in a number of alternative organic solvents, for example DMF, THF, N-methyl-2-pyrrolidone, and DMSO, hence using electrospinning to fabricate conductive fibrous scaffolds has become very popular. Furthermore, the range and adaptability of incorporating various other polymeric materials, or nanofillers, specific molecules for drug delivery and tailorability of the final scaffold design (core–shell, random fibrous, or anisotropic fibre alignment), have

made electrospinning a very attractive approach for fabricating PANi scaffolds for various applications. For example, in the study carried out by Hatamzadeh *et al* electrospinning was elegantly used for blending PEGs-b-PANi with PCL to fabricate electrically conductive nanofibrous scaffolds for TE applications [67]. Similarly, Zhang *et al* fabricated a sophisticated aligned core-sheath composite non-fibrous structure of PANi as core containing nerve growth factor (NGF), which was blended with poly(L-lactic acid-co-3-caprolactone)/silk fibroin (PS) as the outer sheath. The fabricated scaffold showed good potential for neural TE [68]. Using the electrospinning process, Simotwo *et al* were able to produce an almost pure free standing PANi film (93 wt% pure). This was possible by blending PANi with ultra-high molecular weight poly (ethylene oxide) (PEO) with PANi in solution, thus enabling the formation of adequate chain entanglements, which are critical for electrospinning. The conductivity of this electrospun mesh, which was proposed as a supercapacitor, was further enhanced by adding 12 wt% CNTs to create nanocomposite PANi/PEO/CNT nanofibers [69].

Bagheri *et al* fabricated PVA/chitosan (CS)/oligoaniline electrospun meshes composed of 15 wt% of PVA and varying amounts of CS/oligoaniline (5, 10 and 20 wt% of oligoaniline) [70]. It was found that the addition of oligoaniline improved the mechanical properties of the electrospun meshes. It has also been reported that the mechanical properties as well as the electrical conductivity of electrospun fibres can be enhanced by performing post processing steps such as annealing [31], crosslinking [19] and doping [34].

2.3.2. Hydrogel processing

The development of conductive hydrogels for a range of TE applications, including heart, skin, muscle, spinal cord, etc, has notably increased as shown in the recent surge in related publications. A recent review by Xu *et al* [71] highlights the most recent research on the topic. Hydrogels are an interesting type of biomaterial for many TE applications, in particular TE of soft tissues, because of their texture, excellent biocompatibility and tunable mechanical properties. Chakraborty *et al* [72] recently developed short-peptide self-assembled nanostructured hydrogels, adding a di-Fmoc based hydrogelator containing the cell-adhesive Arg-Gly-Asp (RGD) fragment, which enabled the formation of a mechanically stable, self-healing hydrogel (denominated Fmoc-K(Fmoc)-RGD molecule). To impart electrical conductivity, PANi fibres were incorporated into the hydrogel structure. A solvent-switch technique was used to fabricate the composite hydrogels. The hydrogels exhibited not only semi-conductivity (the highest value of current measured was approximately 0.4 μA), but furthermore exceptional antibacterial activity and DNA binding capacity. The proposed application of the scaffold was cardiac TE and cardiac cells grown on the surface of the hydrogels formed a synchronized monolayer. A study by Sun *et al* investigated the *in vitro* and *in vivo* biocompatibility of a myo-Inositol-1,2,3,4,5,6-hexakisphosphate (Ins P6) gelated PANi hydrogel. Ins P6, commonly known as phytic acid, was first described as an abundant form of phosphorus in plant seeds and other plant tissues, however it is also present in eukaryotic cells. In the study, electrospinning was used to fabricate a PCL scaffold. Subsequently, two aqueous solutions, one of ammonium persulfate (APS) (solution A) and the other of aniline monomer and phytic acid (solution B) were prepared. These were mixed together and used for dip coating the PCL scaffolds. The conductive coating applied using a dip coating technique on the PCL scaffold was found to be stable. The addition of PANi had a pronounced effect on both mechanical characteristics and cell adhesion and proliferation of rat endothelial progenitor cells [73].

A variation or subform of hydrogels are aerogels, in which the liquid phase of a gel is replaced by a gaseous phase, which leads to a large degree of shrinkage, usually stronger/stiffer structures and high porosities. Hosseini *et al* fabricated an aerogel composed of bacterial cellulose (BC)/silver NPs/PANi for soft TE [15]. The composite aerogels were fabricated using varying concentrations of $AgNO_3$ with the addition of PEG in either a 0.01 M and 0.25 M HCl solution with homogenous mixing of each component for a period of 15–60 min between each addition. The fabrication of dynamic conductive hydrogels is also interesting because they possess dynamic and reversible crosslinks, which enables the breaking and reforming of the reversible linkages. Therefore, they are able to provide dynamic environments for optimized cellular functions while maintaining matrix integrity [15].

2.3.3. 3D printing

3D printing of hydrogels for biomedical applications has gained increasing interest in recent years, whereas the 3D printing of conductive hydrogels is still a relatively new development. Three different methods of 3D printing are usually considered, i.e. bioplotting, light-based printing and ink jet printing. In bioplotting, the conductive hydrogel or its precursors are fed through a nozzle using for example pressurized air. The pressure causes the hydrogel to flow and deposit in the desired arrangement. Usually, crosslinking is used directly afterwards to avoid distortion of the structure. On the other hand, in light-based printing, e.g. stereolithography methods, a light reactive hydrogel precursor is illuminated by a travelling UV light or laser to induce crosslinking. When using inkjet methods, microvolumes of the hydrogel or precursors are

deposited on a printing stage by deformation of a piezoelement, which leads to the deposition of small droplets [53].

Wibowo *et al* used a bioplotter to plot PCL/PANi scaffolds with varying concentration of PANi up to 2%. The melted PCL and the PANi powder were mixed using melt blending for at least 30 min and subsequently cooled and cut into small pieces, which could be loaded into the printing chamber. The blend was further homogenized by means of rotation of the screw during extrusion in the printing process. The scaffolds were then printed using a nozzle with a diameter of 330 μm. The scaffold was printed using a scaffold architecture designed using a CAD software with an overall size of 30 mm $\times$ 30 mm $\times$ 3.36 mm, a fibre spacing of 660 μm, a slice thickness of 280 μm, and a 0°/90° lay down pattern. The printing parameters used were: deposition velocity of 20 mm s^{-1}, material chamber temperature of 90 °C, an extrusion pressure of 6 bar, and a screw rotation velocity of 15 rpm. The results clearly showed that the conductivity increased with increasing content of PANi, and it was within the range desirable for bone TE applications, however higher concentrations of PANi (e.g. 1 and 2 wt% PANi) led to a slight cytotoxic effect [74].

2.4. Biocompatibility of PANi

A major development concerning the biocompatibility of PANi has been the decision by the World Health Organisation (WHO), International Agency for Research on Cancer, to classify aniline (An) as a probable carcinogen to humans (Group 2A) [75]. This means that to date, there is insufficient evidence of carcinogenicity in humans but adequate evidence in experimental animals. This classification puts aniline under the same category of various food products such as red meat, processed food and diet soda drinks that contain phenylamine. In fact, historically, the biocompatibility of PANi had been debated over two main reasons. First, in relation to the use of monomers like aniline and reaction by-product like aniline dimer (AD) benzidine, that is formed during the synthesis, which is an aromatic amine and hence suspected to be carcinogenic. However, the use of An still remains very contentious as aniline and its derivatives have been successfully used in a wide variety of applications such as precursors in the polymer industry for the synthesis of polyurethane, which is routinely used for biomedical applications, e.g. as artificial vascular graft prosthesis. Over the years it has been well established that the cytotoxicity of PANi occurs as a result of the by-products of the polymer synthesis rather than due to PANi itself and that its biocompatibility can be significantly enhanced by repeated purification, washing, de-protonation and re-protonation cycles [37, 76]. In the study carried out by Kašpárková *et al* [77] the cytoxicity of different precursors: aniline (An), aniline hydrochloride (AnH), and APS on mouse embryonic fibroblast NIH/3T3 cells was studied. APS concentrations below 0.1 mg ml^{-1} were not cytotoxic for NIH/3T3 cells, and concentrations of 0.25 and 0.5 mg ml^{-1} showed only mild cytotoxicity while for AnH at concentrations between 0.75 and 0.025 mg ml^{-1}, no influence in cell viability with 100% cell survival was observed. Concentrations above 1 mg ml^{-1} exhibited mild to moderate toxicity. No toxicity was observed for concentrations below 0.25 mg ml^{-1} [77]. This study further confirmed that the biocompatibility of PANi can be greatly impacted and improved by choosing the right reactants during synthesis and furthermore by choosing the right dose, as the reactants show a dose dependent cytotoxicity.

The second reason for the hesitancy in the biocompatibility of PANi arose from its non-biodegradable nature. Although numerous artificial biostable prosthesis are routinely used in humans in a clinical setting, in the absence of any long-term *in vivo* study, it remain to be investigated, if the long-term presence of PANi in the body could trigger foreign body response and chronic inflammation. In the past few years numerous studies have been undertaken to make PANi biodegradable and thus improve its biocompatibility. One common approach to this is to combine PANi with other biodegradable and biocompatible biomaterial to form blends and composite systems for various biomedical applications including TE. For example, in the study carried out by Ostrovidov and colleagues [78], composite nanofibrous scaffolds of PANi and gelatin were fabricated as potential scaffolds for skeletal muscle TE. The seeded C2C12 cells were found to have enhanced myotube formation and maturation on the composite scaffolds when compared to gelatin nanofibers. Also in the myotubes, the speed and the rate of A band formation were increased, indicating a better intracellular organization. Similarly, an improvement in the colocalization of the DHPR and RyR receptors in the myotubes were also observed which demonstrated an improvement in the myotube maturation and E-C coupling. Furthermore, when the myotubes were electrically stimulated, an improvement in its functionality was also observed because of the increase in calcium transients and contractions with higher amplitude and regularity. This study therefore demonstrated that gelatine—PANi composite nanofibers can successfully provide topographical and electrical cues to direct skeletal muscle cell organisation leading to improved cellular maturation, functionality and tissue formation [78]. However, lately efforts have been made to combine PANi with biomaterials which enhance not only PANi biodegradability but also improves its solubility in water, thereby enabling its processing using less toxic solvents and enhancing as a result, its biocompatibility. Shaabani and Sedghi [6], created a water soluble

composite (PANi/CSG) by synthesising PANi with chitosan biguanidine (CSG) which is a more hydrophilic derivative of CS. The PANI/CSG composite was then successfully incorporated into self-healing polyurethane to form scaffolds for bone TE applications. Here, the self-healing mechanism of the polyurethane is based on the thiol/disulfide redox dynamic exchange reactions and was seen to be enhanced by the incorporation of PANI/CGS. When tested with hADSCs, the scaffold supported the growth and proliferation of the cells and also matrix mineralisation. In fact a significant enhancement in the gene expression level of collagen Type 1 (COL-1), alkaline phosphatase (ALP), Runt-related transcription factor 2 (RUNX2) and osteocalcin (OCN) suggested that CP (PANI/CGS) incorporation can up-regulate hADSCs differentiation, ECM mineralization and maturation, thus demonstrating its potential for the healing of cancellous bone defects [6].

Doping is a critical step in the conductivity of PANi therefore, huge efforts have concentrated on understanding how various dopants affect the biocompatibility of PANi, PANi based matrices (blends and composites) and how their biocompatibility can be improved. Routinely, strong acids have been used for the doping (protonation) of the PANi backbone. Here, there is a risk of this acidic dopant leaching from the PANi matrix under aqueous environment, which can cause a local acidic environment surrounding the PANi component, resulting in some level of toxicity to the surrounding biological environment. For example, camphor sulfonic acid (CSA) has been frequently used as a dopant and can maintain the conductivity of PANi over a wide pH range (0–12) [79, 80]. However, the leaching of CSA from PANi has been reported to cause a localised acidic environment that is toxic for normal cell activities. Therefore, replacing such acidic and toxic dopants with bioactive substances is an interesting advancement. In this context, Daraeinejad and Shabani [81] studied the use of a bioactive substance, Taurine (2-aminoethane sulfonic acid) (Tau), as a potential dopant for PANIi [81]. Tau is an amino acid containing member of the sulfonic acid family and has been shown to promote proliferation, differentiation, and survival of neural stem cells in the brain [82] and to prevent oxidative damage on incisional skin wounds [83]. Direct comparison of the effect of Tau doped poly(ethersulfone)/PANi fibrous scaffold (PPT) and commonly used CSA doped poly(ethersulfone)/PANi scaffold (PPC) on the biocompatibility of 3T3 fibroblast cells was carried out. *In vitro* cell cytotoxicity studies were performed by exposing and growing the 3T3 cells in the PPT and PPC extracted medium (PPT and PPC scaffolds were incubated in Dulbecco's Modified Eagle Medium (DMEM) for 1, 3 and 7 d to extract the leachate in the medium). The cell viability of 3T3 cells in the PPT medium was significantly higher (>90%) for all the different concentrations of extracts (100%, 50% and 25%) when compared to the PPC medium (<80%). This study clearly showed that Tau is less toxic than the commonly used CSA, however the downside to its use is that the conductivity of the PPT scaffolds was lower than that of the PPC scaffolds. A previous study has shown that with the increase of the dopant chain length, the solubility of PANi increases. Therefore, as CSA chain length is longer than that of Tau, PPC scaffolds were more soluble, which increased the final content of PANi in the scaffolds and hence they were more conductive than PPT scaffolds. Also, over a period of seven days, as the scaffolds were incubated in the culture medium, the conductivity of PPT scaffolds had reduced from $0.5 \pm 0.08 \times 10^{-5}$ S cm^{-1} to $4.3 \pm 0.29 \times 10^{-7}$ S cm^{-1}, whereas for the PPC scaffold the conductivity had reduced from $3.7 \pm 0.2 \times 10^{-5}$ S cm^{-1} to $8.3 \pm 0.35 \times 10^{-6}$ S cm^{-1}. The authors reported that the higher reduction in the conductivity of the PPT scaffolds is due to PANi de-doping time not being the same for different dopants and being affected by the smaller molecular size and water solubility of the dopant. Tau, being smaller in size than CSA and highly soluble in water, is easily separated from the PANi chains and is released into the medium. In this study, although the authors have highlighted the role of dopant molecular size and solubility as factors affecting the de-doping of PANi scaffolds, the pH of the environment in which the scaffolds are immersed is crucial in the de-doping process. Nevertheless, this study clearly highlights an important unresolved issue, which is the need to process highly conductive PANi matrices and the maintenance of their conductivity at physiological pH while using less toxic dopants for improving biocompatibility.

A smart approach to address this issue was proposed by Almasi *et al* [84], in this work instead of using any acid donor and doping route, the dopant in the form of graphene oxide (GO) nanosheet was incorporated into the PANi matrix to form GO doped PANi [84]. The existence of long pair of electrons on the oxygen atoms allows GO nanosheets to act as dopant for PANi [85]. When the GO particles are reduced by PANi during the doping process, the GO NPs get converted to nanosheets that have higher conductivity due to π-π stacking and cation-π interactions [86]. Moreover, the presence of functional groups containing oxygen atoms imparts hydrophilic property to GO NPs making them soluble in water and in a number of other organic solvents. Furthermore, GO NPs at concentration of 1 mg kg^{-1} of body weight were found to have no pathological effect [87]. Therefore, in this study by Almasi *et al* [84], *in situ* polymerisation of aniline monomers was carried out in the presence of GO particles. The doped PANi solution containing the GO nanosheets was dried at 60 °C and then combined with polyacrylonitrile (PAN) to fabricate electrospun fibrous composite scaffold (PAN-PANi-NP). FTIR analysis confirmed the presence of electrostatic/hydrogen bonds between GO nanosheets and PANi. Satellite cells from skeletal muscle showed good adhesion, growth

and proliferation on the PAN-PANi-NP as opposed to the bare PAN-PANi scaffold and TCP control. This also correlated well with the higher expression of protein markers, such as Troponin I and Troponin T, both belonging to the contractile apparatus in the PAN-PANi-NP scaffolds, in comparison to that of the bare PAN-PANi scaffold and TCP control [84]. Although these cell culture and gene expression studies do indicate that the incorporation of GO in PANi as a dopant was successful in maintaining its interactions in physiological pH, more time dependent conductivity analysis of the scaffolds would have provided more insight into the doping efficiency of GO and overall conductivity of the scaffold. It is well established that the conductive nature of PANi matrices plays a key role in the improved interactions with cells particularly of excitable tissues such as skeletal muscle, nerve, cardiac tissue, and bone. Another important development has been the investigation of the effect of dopants on the biocompatibility of PANi based scaffolds via their influence on the surface properties (roughness) and wettability of the scaffolds. For example, Liu *et al* [88] investigated how various dopants affect the surface properties, roughness and wettability of the doped scaffold (PANi/PLA composite nanofibres), and thus how they impact the scaffold interactions with cells, in this case human osteosarcoma (HOS) cells. Spun PLA nanofibers were immersed in PANi prepared by *in situ* oxidative polymerisation in the presence of different inorganic acid dopants (HCl, HA; H_2SO_4, SA; $HClO_4$, PA) to form PANi/PLA-HA, PANi/PLA-SA and PANi/PLA-PA composite nanofibers, respectively. Morphological analysis revealed that PANi/PLA composite nanofibers were successfully coated with PANi while maintaining good fibre morphology and porous nanofibrous structure. Atomic force microscopy (AFM) results showed that the surface roughness values of the composites were (PANi/PLA-HA, Ra $= 0.308$ μm; PANi/PLA-SA, Ra $= 0.315$ μm and PANi/PLA-PA, Ra $= 0.332$ μm) higher than the roughness values of the control PLA (Ra $= 0.278$ μm). Similarly, PLA is hydrophobic ($\theta = 112°$), whereas the wettability of the composites had improved due to the presence of PANi: PANi/PLA-HA, $\theta = 61.6°$, PANi/PLA-SA, $\theta = 36.7°$ and PANi/PLA-PA $= 37.2°$, respectively. These increases in surface roughness and wettability of the PANi/PLA composite scaffolds had a positive effect on the growth of HOS and were higher in the composites when compared to the control PLA nanofibers. The cell viability, evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-(diphenyltetrazolium bromide) (MTT) assay, showed that the cell activity gradually increased over the five day study period and was highest for the PANi/PLA-PA composite, which had the highest roughness values amongst all composites. It is well established that cells prefer rough surface topography in dimensions mimicking their native ECM, here the authors indicated that the increase in the surface roughness enhanced the surface area and thereby provided an increased number of growth sites for cells promoting cell adhesion. Similarly, the PANi/PLA composites doped with different acids resulted in improved wettability, surface energy and polarity, which further contributed to the increase in adhesion, growth, migration and proliferation of the HOS cells. This higher compatibility of HOS for the composites also correlated with the increased expression of early osteogenic markers, studied over seven days, enhanced by the presence of PANi [88].

Various other strategies have also been used to improve the biocompatibility of PANi. Some of these have focussed on the functionalisation or incorporation of bioactive molecules in the PANi matrix. For example in the studies carried out by Li *et al* [89], functionalisation of PANi by grafting Tyr-Ile-Gly-Ser-Arg, and Arg-Tyr-Ser-Gly-Ile onto the PANi backbone was seen to enhance proliferation of neuronal PC-12 cells, and promoted neurite extension and neuronal network formation without the necessity of adding NFG [90]. Similarly, when cyclic Arg-Gly-Asp-D-Phe-Lys containing the RGD peptide sequence was grafted on ATQD, an electroactive oligomer derived from PANi, it enhanced the adhesion of PC-12 cells on the scaffold and also improved cell proliferation rate and induced neurite outgrowth from the cells [91]. In another approach, polyaniline nanofibers (PNFs) were functionalised with glutaraldehyde, which successfully incorporated polar hydroxyl (–OH) and aldehyde (–CHO) groups into the PNFs, thereby increasing its surface energy, polarity and wettability. The surface functionalised, SF-PNFs were also seen to support the growth of PBMC with an increased cell viability, which was twice that of the control and the pristine samples [51]. The morphology of PANi has also been shown to affect its biocompatibility. It was reported that PANi and poly(L-lactide-co-ε-caprolactone) (PLCL) composites exhibited different levels of cytotoxicity to PC-12 cells when in powder or fibre forms. In the fibre form, PANi particles were present not much on the surface but rather embedded inside the PLCL matrix on which the cells were attached, whereas in the powder form, the cells were in direct contact with PANi [92]. In the study by Saracino *et al* [93], PANi was synthesized in the form of nano-needles (PnNs) and electrospun into nanofibers containing gelatin. The nano-needle shape of the PANi was found to enhance percolation threshold efficiency by enabling formation of more contact points and percolative conductive pathways in the presence of lower amounts of the conductive phases, thereby improving the biocompatibility of the scaffold, as it meant that lower amount of PANi and dopant were needed for the fabrication of the scaffold. The presence of PnNs in the scaffold supported the adhesion and growth of the seeded astrocytes (brain glial cells) over time and its presence in the aligned fibres also enhanced the alignment of the astrocytes, as opposed to aligned fibres without PnNs, thus supporting actin

cytoskeleton rearrangement and focal adhesion complex without causing relevant effects on the active/passive bioelectrical properties of the astrocytes [93]. Another interesting development has been the fabrication of biocompatible PANi based hydrogel systems that are capable of cell encapsulation for bio-printing. Sawyer *et al* [94] fabricated electrically conductive gelatin methacrylate (GelMA)-PANi hydrogels by incorporating doped PANi solutions in GelMA pre-polymer solution. The incorporation of clusters of PANi within the GelMA matrix increases the electroconductivity of the composite gel, while at the same time maintaining the osteoid-like soft mechanical properties that enable three-dimensional encapsulation of living cells. The viability of human osteogenic cells encapsulated within GelMA-PANi hydrogels was similar to the GelMA scaffolds without PANi. Cells within GelMA-PANi were capable of depositing mineral phase within the hydrogel matrix after being chemically induced for two weeks, although the total mineral content was lower as compared to pure GelMA. The authors were also able to print GelMA-PANi containing encapsulated cells in user defined geometries. This study has therefore opened avenues for fabricating electro-active biological circuits which can be actively stimulated with external electrical cues for better understanding of the effect of electrical signals on cellular functions [94].

2.4.1. In vivo *studies*

Over the years tremendous advancements in different aspects of PANi, ranging from its synthesis, processing and fabrication have been made, that have further expanded the research on the application of PANI in wide ranging areas. All these studies have incorporated assessment of the biocompatibility of PANI and most often these have been carried out at the *in vitro* level looking at the adhesion, proliferation, differentiation of target cells on PANi based surfaces and their gene expression. Certainly, a plethora of cells have been studied, all contributing towards the understanding of the biocompatibility of pure PANi or PANi in combination with other polymer materials. At an *in vivo* level, Kamalesh *et al* [95] demonstrated the *in vivo* biocompatibility of PANi. Subcutaneous implantation in male Sprague Dawley rats for up to 90 weeks showed no signs of toxicity or abnormality in the surrounding tissues with no elicitation of undesirable inflammatory response observed [95]. Although this study was positive and could be considered a real game changer, reservation still existed surrounding the *in vivo* biocompatibility of PANi as a prior study by Wang and colleagues had reported some inflammation and fibrous tissue encapsulation of the implanted PANi in Sprague Dawley rats [96]. On the other hand in the study carried out by Mattioli-Belmonte *et al*, the *in vivo* response to pure PANI in female Sprague-Dawley rats after four weeks of implantation was considered acceptable as no major inflammatory reaction or tumour formation was observed [97]. Clearly, these initial *in vivo* studies have indicatd that more understanding of the *in vivo* response that PANi provokes had to be ascertained, as different factors from a materials standpoint (starting material, doping, post processing, combination with other materials and final scaffold/matrix morphology etc) impact biological interactions.

In fact, this very effect of dopant on the biocompatibility of PANi was highlighted in the study carried out by Mawad *et al* [98]. Phytic acid is a good ionic conductor and a natural molecule that is widely available in plant seeds and plant tissues like bran, being also present in eukaryotic cells. Phytic acid influences biological functions, such as signal transduction, cell proliferation, cell differentiation, exocytosis, antioxidants, mRNA transport and DNA repair [99–102]. Phytic acid was therefore used as a biocompatible dopant to fabricate electronically stable PANi based scaffolds (PANi patch or C-patch) by growing PANi on the surface of a CS film. The strong chelation between the phytic acid and CS resulted in the patch retaining electroactive properties, a relatively low surface resistivity (35.85 ± 9.40 kilohms per square), and oxidized form even after two weeks of incubation in physiological media. *In vivo* studies were performed using adhesion of the C-patches without the use of sutures on the heart tissue of Sprague-Dawley rats. Echocardiography results showed that the adhesion of the patches (both non-conductive and conductive) had no significant effect on global heart function. A small but significant increase in the ejection fraction (EF) and fractional shortening of hearts with C-Patches could be observed in comparison to the control group (without patches). The relaxation properties of the heart were unaffected by either patch. This result suggested that the patches did not inhibit the contractile properties of the heart even though they exhibit a higher Young's modulus than that of the typical adult myocardium. Overall, the observation was optimistic as the C-patch did not induce any proarrhythmogenic activities in the heart [98]. A recent report of an *in vivo* study has been very promising, in which EST of conductive hydrogel prepared by means of copolymerization of N-acryloyl glycinamide (NAGA) with quaternized chitosan-g-polyaniline (QCSP) was shown to heal chronically infected wounds. The QCSP-g-PNAGA hydrogel had a comparable conductivity to that of human skin and was tough due to the dual hydrogen bonding formed from the PNAGA segments. *An in vivo* assay in diabetic rats showed that EST from the conductive hydrogel was more effective in promoting healing of infected wounds than the conventional EST via rigid electrodes. For the EST + hydrogel group, by day 7 the trauma size was 18% smaller than that of the control and was almost healed by day 14 (figure 6). EST of the conductive hydrogel significantly enhanced fibroblast migration resulting in the healing of the wound.

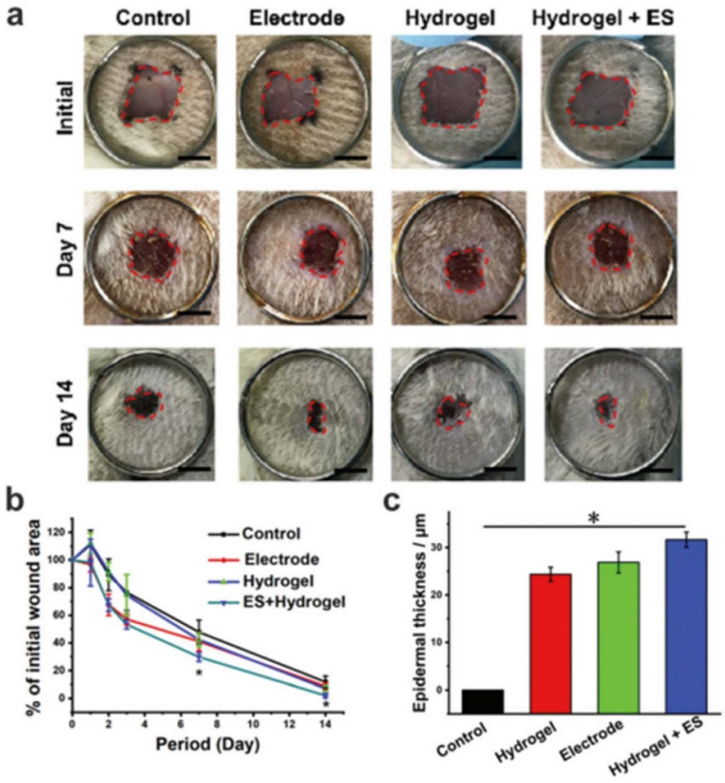

Figure 6. (a) Digital photographs of wound size and appearance for the four experimental groups, i.e. control, electrode, hydrogel and hydrogel + EST over 14 days. Scale bar = 5 mm. (b) Graph illustrating the percentages of initial wound area for the experimental groups over 14 days. *Indicates a significant difference ($p < 0.05$) compared with the control group on the same day. (c) Thickness of regenerated epidermal layers at wound area on day 14. *Indicates a significant difference. Reproduced from [103] with permission from the Royal Society of Chemistry.

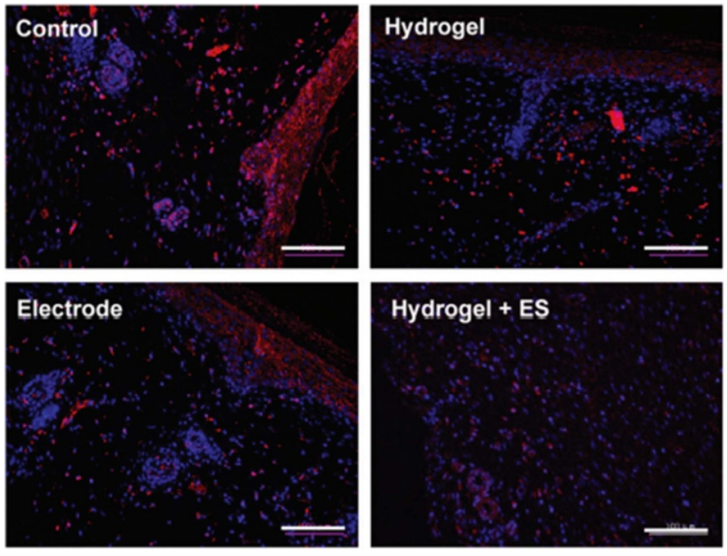

Figure 7. Fluorescence immunohistochemical analysis of the regenerated tissues on day 14 at the wound sites with CD68 (macrophages) staining. The red fluorescence indicates macrophage, and the blue fluorescence indicates cell nuclei. Scale bar = 100 mm. Reproduced from [103] with permission from the Royal Society of Chemistry.

Moreover, the fact that PANi is inherently antibacterial helped in fighting wound infection promoting its healing. Immunohistochemical staining with the CD68 antibody showed large presence of macrophages in the control group when compared to that of the hydrogel and the electrode groups, while the EST + hydrogel group had the lowest presence of macrophages (figure 7). This study further demonstrated that the EST + hydrogel group did not induce any inflammatory effect and thus the materials were biocompatible and considered to be safe to use as a treatment approach to heal chronic wounds [103].

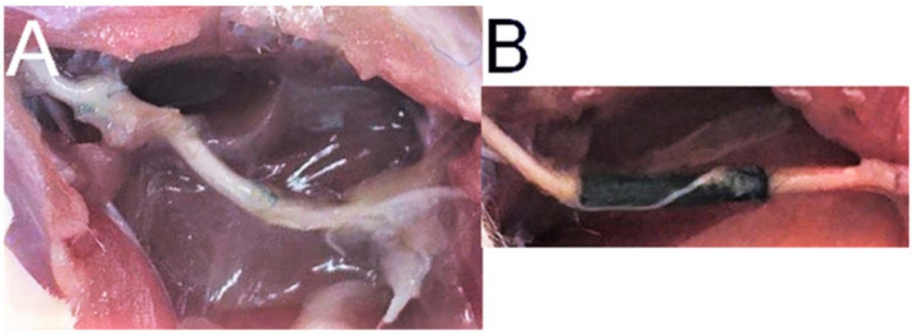

Figure 8. Six months after the intervention, the sciatic nerve was excised for further analysis. (A) Group B—sciatic nerve reconstructed with autograft after six months, (B) Group D—sciatic nerve reconstructed with P(LLA-CL)-COL-PANi conduits after six months. Reproduced from [17]. CC BY 4.0.

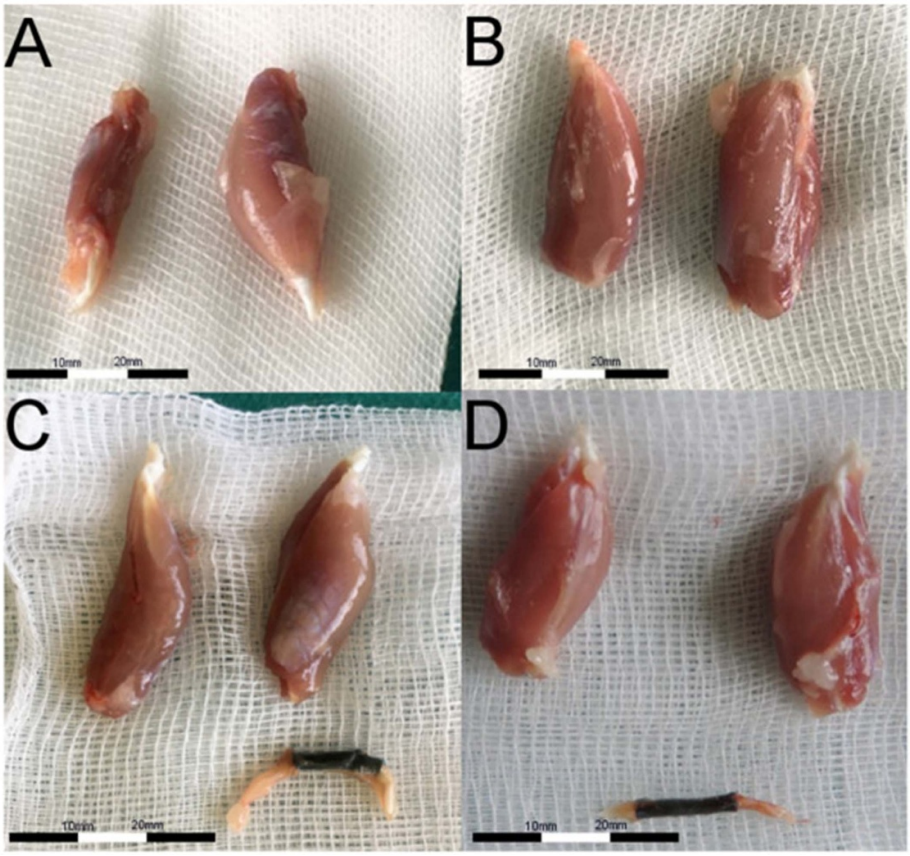

Figure 9. Digital photographs (postoperative) of the gastrocnemius muscle excised after 6 months from intervention—muscle from an operated limb (on the left side), muscle from the side after reconstructive procedure (on the right side).
(A) Control group—nerve gap remained, the muscle was innervated and decreased its size vs. unoperated limb on the right.
(B) Autograft—muscle size is comparable. (C) P(LLA-CL)-COL-PANi conduit integrated into the sciatic nerve (bottom), which substantially preserved gastrocnemius size (left). (D) P(LLA-CL)-COL-PANi conduit was enriched with ASC cells, which prevented the muscle from atrophy (left vs. right)]. Reproduced from [17]. CC BY 4.0.

Another promising study was carried out by Dębski and colleagues [17], who looked into the efficiency of poly (L-lactic acid)-co-poly(ε- caprolactone), collagen (COL), PANi, P(P(LLA-CL)-COL-PANi) fibrous scaffold as a nerve conduit for the treatment of peripheral nerve gap. A total of 28 inbred male Lewis rats were subjected to sciatic nerve transection and an excision of a 10 mm nerve trunk fragment was made. In Group A, the nerve gap remained untouched; in Group B, an excised trunk was used as an autograft; in Group C, nerve stumps were secured with P(LLA-CL)-COL-PANi conduit; in Group D, P(LLA-CL)-COL-PANi conduit was enriched with ASCs. After six months of post implantation (figures 8 and 9) qualitative and quantitative muscle analysis revealed that the conduits reconstituted nerve continuity and prevented muscle atrophy, which was seen in the control group. Also, in the conduits containing adipose

derived stem cells (ASCs) the muscle mass ratio preservation with relation to the autograft was 87%, whereas for the conduit alone it was 96%. However, in the ASC rich conduit, the highest number of nerve fibres was produced. The developed tubes possessed good strength and elasticity to avoid post implantation chronic compression (collapsing of the tube). Overall, the study demonstrated that the P(LLA-CL)-COL-PANi conduits were biocompatible and provided effective support for axonal regeneration in peripheral nerve gap reconstruction, as demonstrated in a rat model [17].

All these *in vivo* studies have shed vital insight into the biocompatibility of PANi and PANi based materials, however most of these studies have so far been carried out in rat models. Therefore, it will be interesting to understand the biocompatibility of these materials in higher animal models like sheep and porcine and for longer implantation time before progressing to first-in-man studies. All artificial prosthesis, when incorporated *in vivo*, elicit a host of responses and increasingly the role of biomaterials specifically their interaction with macrophages, in determining the potential fate of the implant via pro-inflammatory and healing pathways, is being recognised. Therefore, more understanding on how these pathways are triggered by the presence of PANi based matrices needs to be gained to further improve PANi *in vivo* biocompatibility.

3. Advantages of PANi

3.1. Biodegradability

Conductive polymers in general, including PANi in its pure form, are non biodegradable. Although long term *in vivo* studies of PANi have not been carried out, its sustained long term presence in the body has potential of inducing a foreign body reaction resulting in scaffold failure and requiring a second surgical intervention to remove the PANi based scaffold. It therefore becomes essential to convert PANi into a biodegradable form especially for TE applications, whereby the PANi scaffold would support tissue remodelling and its rate of degradation should be tailored to match new tissue formation.

The most commonly used route to develop biodegradable PANi involves combining it with other biodegradable biomaterials to develop blends and composite systems. For example PANi has been combined with a whole range of both synthetic and natural polymeric materials such as PCL, poly(lactic acid) (PLA), poly(3-hydroxybutyrate) P(3HB), CS, gelatin and cellulose among others. An interesting study was carried out by Eftekhari *et al* [104] who combined PANi with CS to develop conductive CS/PANi composite hydrogel with PC12 cell-imprinted topography, as a potential substrate for neural priming of ASCs. The scaffolds were subjected to *in vitro* degradation under static and shaking conditions in PBS for 35 days and it was seen that the CS-substrates degraded at a faster rate than that of the CS-PANi substrates. After two weeks, the CS gels were able to preserve approximately 53% and 60% of their initial mass in both the shaking and static conditions, whereas for the composite hydrogel only 34% and 30% of weight loss was observed under similar conditions. PANi is hydrophobic in nature when compared to CS and therefore when incorporated into the CS matrix, it acts as a barrier against water penetration resulting in a slower weight loss in the CS-PANi substrates [104]. Studies with blends and composites of PANi have reported certain weight loss at *in vitro* level, however, it is still anticipated that once the combining biodegradable material has completely degraded, the remaining PANi will not degrade and if implanted it would continue to persist in the body. This issue was highlighted in the work carried out by Wang *et al* [105]. In this study, zein biodegradable microtubes were coated with conductive PANi NPs to develop conductive conduits for repairing peripheral nerve injury. After two months of implantation in adult male SD rats the PANi coated conduits showed significant improvement in the recovery of proximal compound muscle action potential when compared to that of the non coated and autograft group. However, after four months of implantation the repairing efficacy of the conduits turned bad as by then the biodegradable zein tube had completely degraded and PANi coating disintegrated into fragments forming debris within and around the regenerated nerve (figure 10) [105]. Therefore, this study indicates a clear potential of PANi as a matrix for supporting tissue regeneration but at the same time it shows the crucial need to impart biodegradability. If the biodegradability could also be tailored, this would further make PANi more attractive for TE applications in particular.

In fact, over the years different chemical approaches have indeed been undertaken to develop biodegradable PANi. Inspired by the seminal study carried out by Zelikin *et al* [106], who demonstrated development of erodible PPy by polymerizing β-substituted pyrrole monomers containing hydrolyzable side segments [106] similar strategies for aniline based conducting polymers (CPs) have been studied [37, 107]. For example, Guo *et al* developed a two-step synthesis approach combining ring opening polymerisation and oxidative coupling to develop biodegradable and conductive diblock or triblock copolymers [108]. Using this approach, a series of degradable and conductive diblock copolymers and networks with different molecular weights of PCL and different aniline tetramer (AT) contents were synthesized. In the first step, AD was used for initiating the ring-opening polymerisation of ε-caprolactone monomer (CL) in the presence of Sn(Oct)2 as a catalyst, resulting in the formation of a diblock AD-PCL copolymer with a controlled structure, having a

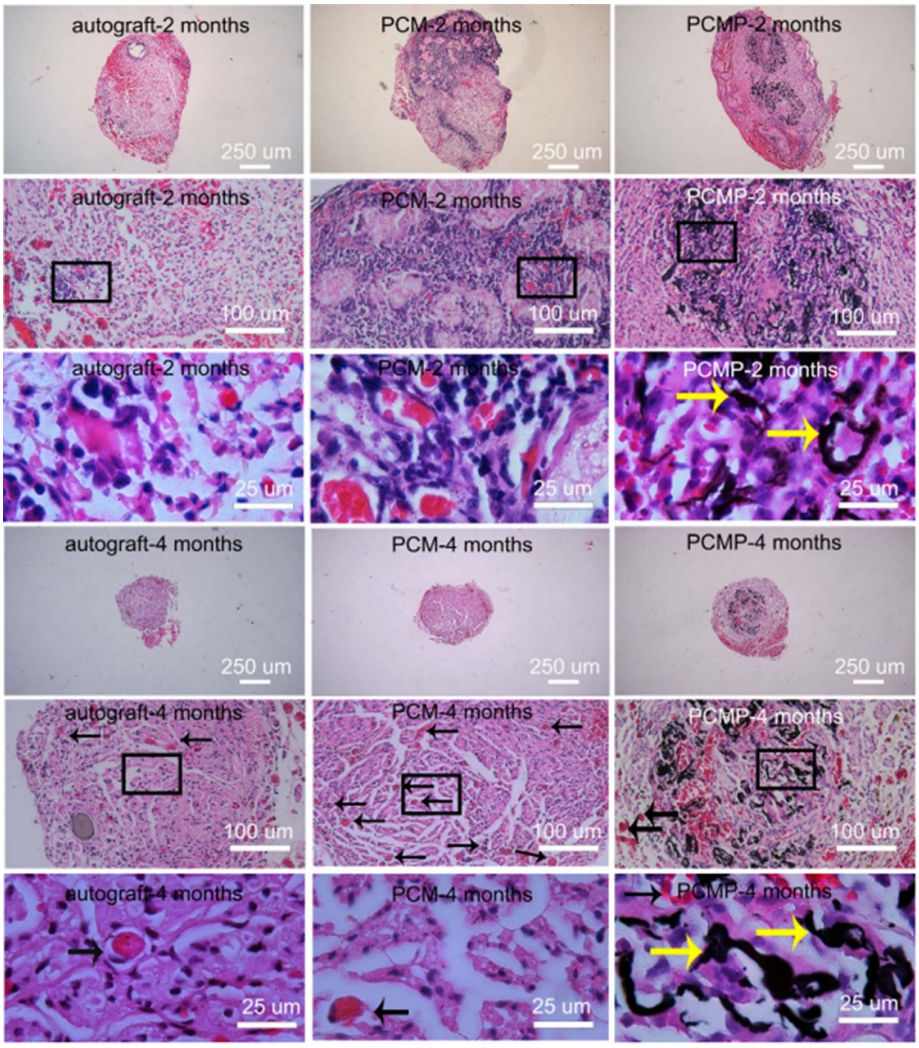

Figure 10. Photographs of H&E-stained regenerated nerve sections in second and fourth month post implantation at 40×, 200×, and 1000× magnification. The region in the panel was magnified. The black arrows mean the regenerated vessels and the yellow arrows mean the PANi [105]. John Wiley & Sons. © 2019 Wiley Periodicals, Inc.

rigid AD segment at the chain end as one block and the long degradable flexible PCL as the other block. The AD group in the AD-PCL was then used for the oxidative coupling post polymerisation modification with AD resulting in the formation of a conductive AT segment at the chain end of the macromolecules [108]. Using the same ring opening and post oxidative coupling, a terpolymer composed of PANi-co-(polydopamine-grafted-poly(D,L-lactide)) [PANi-co-(PDA-g-PLA)] was synthesized by Massoumi *et al* [109]. The authors chose to use PDA owing to its biocompatibility, good adhesive features and presence of chemical moieties that allows for post-modification. Similarly, PLA was used because of its inherent physicochemical as well as biological properties, like biocompatibility, bioresorbability and acceptable mechanical properties. Combining these two different polymers with PANi would result in the formation of a terpolymer that is conductive, bioadhesive, biocompatible and biodegradable, properties that would be ideal as a scaffolding material for TE applications. First, a one-step chemical oxidation polymerisation of aniline and dopamine monomers under acidic conditions to produce PANi-co-PDA was carried out. The lactide monomers were then grafted onto the PDA segment by ring-opening polymerisation. The resultant terpolymer was electrospun to produce a conductive porous nanofibrous scaffold, which demonstrated excellent physicochemical properties (mechanical strength, conductivity, electroactivity, wettability, and morphology). At the end of seven weeks of *in vitro* degradation studies, the terpolymer had lost a total mass of 49.3% in physiological medium, pH 7.4 [109].

In another study, the carboxyl group on phenyl/carboxyl-capped aniline pentamer was crosslinked to the hydroxyl group on the end of the 4-armed P(D,L-Lactide) to form 4-armed P(D,L-lactide-co-aniline pentamer) (4a-PLAAP) [110]. The 4a-PLAAP was then doped using varying concentrations of chondroitin sulfate and casted into films. When subjected to *in vitro* degradation studies for approximately 24 days in PBS, neural basal medium and neural basal medium containing lipase, the films showed maximum weight

loss (53%) in the enzymatic medium whereas for the basal medium weight loss was 36% and for PBS 24%. These results also demonstrated that by carefully incorporating monomers, in this case PLA, the 4a-PLAAP could undergo both enzymatic cleavage and hydrolysis. Also, in an *in vivo* setting, most likely the polymer would degrade into small molecules of PLA and aniline oligomers (AOs) [110]. This study cleverly demonstrated that by selectively integrating chemical moieties onto the PANi backbone, the resulting PANi copolymeric matrix can undergo both enzymatic and hydrolytic degradation. These small fragments and debris would then subsequently be consumed by macrophages or degraded by a lysosomal enzyme, and then eliminated out of the human body as metabolites [107, 110].

The analysis of the literature thus indicates a major focus on developing PANi copolymers by incorporating biodegradable functional groups. Also, increasingly, AOs such as aniline trimer (AT), tetramer (TA) and pentamer (AP) are being used as they provide new opportunity to design and synthesize well-defined and well-characterized conductive aniline polymers with defined functionality and properties [107].

3.2. Antibacterial activity

Preventing and treating bacterial infections have always been an important aspect in determining the long-term success and performance of implanted artificial prosthesis. As bacteria and their subsequent biofilm formation are showing an alarming increase in their resistance to current antimicrobial drugs, there is therefore an urgent need to discover and develop new chemicals with high antimicrobial activities. In this context one area of active research is given by polymers with potential antimicrobial properties and one such attractive candidate is PANi. This is because of its relatively easy synthesis that does not require high level of safety and specialised equipment, electroactivity and easy fabrication to suit wide ranging applications that benefit from its antibacterial properties, such as electroactive antibacterial and antifouling biomaterials for TE applications, antibacterial coatings and antifouling ultrafiltration membranes.

The earliest study on the potential application of PANi for its antibacterial property was not rooted on any exploration for biomedical application but rather on developing antibacterial textiles. Seshadri and Bhat [111] looked into PANi as a potential antimicrobial agent as it contains both nitrogen and chloride ions, moieties similar to those present in quarternary ammonium salts and halamines that were assessed for their antibacterial properties. The conductive cotton textile was prepared by the *in situ* polymerisation of aniline and was found to be 95% effective against Gram-positive *S. aureus,* 85% effective against the Gram-negative *E. coli*, and 92% effective against the fungus *C. albicans*. The authors explained the observed antibacterial effect of PANi as a result of the presence of charged states of N and Cl ions in the PANi chains, which attack the bacterial cell walls [111]. These promising results paved the way for the exploration of PANi as potential antimicrobial agent. This study was then followed up by Shi *et al* [112] who observed a 100% reduction in the growth of *E. coli* and *S. aureus* after 24 h of growth in cast films of PANi- polyvinylalcohol (PVA) while pure PVA did not show any antibacterial behaviour. Here again, the authors credited the antibacterial efficacy of the PANi-PVA films to the electrostatic adherence between the PANi molecules and the bacteria considering that they carry charges of opposite polarity, which causes the bacterial cell wall to rupture resulting in bacterial death [112]. Dhivya *et al* [113] have provided an explanation for the antibacterial mechanism of PANi. It is now well understood that when exposed to bacteria, PANi binds to cells because of electrostatic interactions, getting anchored to the cell wall at several sites. Following this, the ion channels present on the cell wall experience a change in the potential gradient, which results in the disturbance of the normal influx and efflux of electrolytes thus disturbing cell permeability. The dopant ions may thus break inside the cell causing damage by interacting with phosphorous and sulphur containing compounds such as DNA and protein [113, 114]. The electrostatic interactions between PANi and the microbial membrane and the disruption of microbial cell permeability are responsible for the antibacterial property of PANi. This mechanism also explains the different antimicrobial response to PANi, which are observed depending on the cell wall structure, particularly as seen between species of Gram positive and Gram negative bacteria. Gram-positive bacteria are composed of a thick layer (20–80 nm) of a three-dimensional rigid structure of peptidoglycan consisting of linear polysaccharide chains that are cross-linked by short peptides [113, 115]. The presence of this rigid and extended cross-linking in the cell wall provides few anchoring sites for the PANi and also makes it difficult for the dopant ions to penetrate. On the other hand in the Gram negative bacteria, the cell wall contains an exterior lipopolysaccharide layer, below which a thin layer (7–8 nm) of peptidoglycan is present [113, 115]. Thus, Gram negative bacteria, which are rich in negatively charged lipopolysaccharides, can attract PANi chains with more positive ions on their backbone. Thus, a higher susceptibility of Gram-negative bacteria for PANi as opposed to Gram positive bacteria is observed. Another antibacterial mechanism of PANi was proposed based on research carried out on its colloidal suspension, which generates hydroxyl radicals (–OH), a strong oxidising agent that can oxidise (decompose) the organic content of the bacteria, thereby killing them in the process. In this case, the electrons and the H^+ on PANi

chains react with dissolved oxygen molecules to form superoxide radical anions ($-O_2^-$). These anions then get converted to hydroperoxy radicals (HO_2^-) which generate hydroxyl radicals (–OH) [116, 117].

In the past few years, as research on the antimicrobial property of PANi gathered more interest, exploration of different approaches to further improve its antimicrobial efficacy has gained momentum. Doping is a critical step in converting the non-conductive EB of PANi into the conductive ES form and the release of the dopant ion from PANi influences its antimicrobial mechanism. Therefore, studies looking into the effect of various dopants on the antimicrobial efficacy of PANi have been carried out. In the study of Dhivya *et al* [113], for example, the group evaluated the antimicrobial property of PANi doped with aromatic nitro compounds such as 2,4,6-trinitrophenol, i.e. picric acid (PANIPI), and 3,5-dinitrobenzoic acid (PANIDN), in addition to the widely used dopant, hydrochloric acid (PANICL) and the polyaniline emeraldine base. The antibacterial test was done against various Gram negative bacteria such as *Shigella dysenteriae, Salmonella enterica, Klebsiella pneumoniae, P. aeruginosa, E. coli*) and Gram positive bacteria such as *S. aureus, Streptococcus pyogenes, Bacillus subtilis, Enterococcus faecalis* and the fungus species *C. albicans*, using the agar well diffusion method with the effects being studied from the diameter of zone of inhibition and minimum inhibitory concentration values. The study found that PANIPI and PANIDN were effective against all tested microorganisms similar to that observed with PANICL. The maximum inhibitory zone diameter of 24 mm at 100 μg ml^{-1} was observed for PANIDN against *E. faecalis*. Also, the zone of inhibition (11–13 mm) obtained for the as-synthesized PANIs against *S. aureus* at 25 μg ml^{-1} was higher than that reported from a previous study for PANi and PANi-mupirocin (7.3 and 7.6 mm) at 30 μg ml^{-1}, which were doped with CSA [118].

It has also been seen that primary doped PANi when doped for the second time using secondary dopants demonstrates increased conductivity. This increased conductivity was explained in one of the earliest studies on secondary doping of PANi, where the authors reported that when treated with secondary dopant, PANi chains expand from a compact coil like structure to a more open, expanded coil like structure resulting in the reduction of π-defects, which then increases conductivity due to intra-molecular effects and greater mobility. Therefore, in this context studies have also been carried out to select secondary dopants to increase the antibacterial activity of PANi scaffolds. For example in the study carried out by Bhattacharya *et al* [119] chloroxylenol was used to replace the toxic m-cresol and p-cresol as secondary dopants to increase conductivity and to also investigate whether its use would enhance the antibacterial activity of the fabricated PANi fibres. As expected, the secondary doped fibres exhibited higher conductivity as opposed to other test samples and control: PC1 fibres (PANi-chloroxylenol-PEO) $= 3.1 \pm 0.23$ S cm^{-1}, PANi-PEO fibres (control 1) $= (9.8 \pm 0.51) \times 10^{-6}$ S cm^{-1}, PC2 (PCL)-chloroxylenol) $= (1.5 \pm 0.37) \times 10^{-7}$ S cm^{-1} and PCL fibres (control 2) $= (6.5 \pm 0.72) \times 10^{-8}$ S cm^{-1}. Interestingly, in the contact killing assay PC1 fibres (PANI-chloroxylenol-PEO) were able to kill both *E.coli* and *S. aureus* completely at 1.0 mg and 0.5 mg ml^{-1}, respectively. Concentrations above 1 mg ml^{-1} of PC2 (PCL)-chloroxylenol) were required for complete killing of *E. coli*, yet the same amount was not effective against *S. aureus*. Being a Gram negative and Gram positive bacteria, the viability of *E. coli* and *S. aureus* varies depending on the charge of the polymer surface that the cell is exposed to and how it interacts with it. Therefore, the observations made with PC1 can be explained by the positively charged backbone of PC1 and the antimicrobial activity of chloroxylenol. Similarly, the results observed with PC2 are only due to the rapid release of chloroxylenol from the fibres as this polymer does not possess any positively charged surface [119].

Composites and blend systems of PANi have also been formulated in the quest for improving its antibacterial effect. These have been developed by incorporating agents such as silver (Ag) NPs, Ag NPs combined with MWCNT, TiO_2 and graphene among others. Jia *et al* [120], for example, carried out a study on PANi composites containing Ag NPs [120]. It was found that the composite system was more effective against *E. coli, S. aureus* and yeast when compared to pure PANi and pure Ag particles. The antibacterial efficacy measured in terms of the growth inhibition rates (R) also increased with increasing concentration of Ag NPs in the composite. Here the antibacterial effect observed with PANi-Ag composites was explained by the presence of the Ag NPs, which create redox imbalance causing bacterial death, and secondly, due to the effectiveness of PANi in stabilising the Ag NPs from aggregation. Therefore, such Ag NPs having large surface area provide more bactericidal effect than the larger or agglomerated Ag NPs [120, 121]. CNTs have been used with PANi as these are known to have cell adherence, demonstrate efficient electrostatic interactions and generate reactive oxygen species (ROS), all properties that can enhance the antibacterial effect. In fact, this very observation was made in the study carried out by Deshmukh *et al* [122], who tested a ternary Ag NPs-supported PANi multiwalled carbon nanotube (Ag NPs–PANi/MWCNT) nanocomposite against *E. coli* and *S. aureus*. The zone of inhibition for the Ag NPs–PANi/MWCNT nanocomposites (20 mm and 19 mm) at 20 μg ml^{-1} for *E. coli* and *S. aureus* were higher than those for Ag NP and Ag NPs–PANi for the same organisms and at the same concentrations [122]. Studies on nanocomposites of PANi with Pt (PANi/Pt) and Pt-Pd (PANi/Pt-Pd) have also been reported in the research work carried out by Boomi *et al* [114] and the

antibacterial effect of the developed nanocomposites was studied for Gram positive bacteria (*Streptococcus* sp. and *Staphylococcus* sp.) and Gram negative bacteria (*E. coli* and *Klebsiella* sp). From the MIC and MBC results, it was concluded that the PANi/Pt-Pd nanocomposite has better antibacterial activity in comparison to PANi/Pt nanocomposite and pristine PANi. Furthermore, the PANi/Pt-Pd nanocomposite was more effective against *Staphylococcus* sp when compared to the rest of the tested microorganisms [114].

Studies have also been conducted on improving the processibility of PANi in the context of hydrogel systems for TE applications whilst also aiming to increase antibacterial efficacy. For example, Zhao *et al* [123] reported the development of a new series of *in situ* forming antibacterial conductive degradable hydrogels using quaternized chitosan (QCS) grafted PANi with oxidized dextran (OD) as crosslinker. *In situ* injectable hydrogels are minimally invasive and can be easily administered to match any shape of damaged tissue. Additionally, hydrogel scaffolds (as artificial ECM) are porous and interconnected structures allowing an exchange of nutrients and metabolites, cell migration and encapsulation of cells and other bioactive molecules for tissue regeneration. Similarly, QCS possesses excellent antibacterial activity however, its cell toxicity remains a major issue hindering its potential application for TE. Therefore, injectable hydrogels of PANi and QCS have been developed, interestingly PANi was used to reduce the cell toxicity of QCS in addition to imparting electrical conductivity [123]. The antibacterial activity of the hydrogel was evaluated using the antibacterial assay for *E. coli* and *S. aureus* revealing that the potency of the hydrogel containing PANi was higher compared to that of the QCS and increased with increasing concentration of PANi. The hydrogel containing the highest amount of PANi, 3 wt% (QCS40P3–Odex) was 95% and 90% effective in killing *E. coli* and *S. aureus*, respectively. *In vivo* studies to assess the antibacterial efficacy of the hydrogel were also carried out by subcutaneous administration in female Sprague–Dawley rats for three days and subsequent culturing the explanted bacterial (*E. coli*) suspension for 18 h. Unlike previous studies where the bacterial suspension was injected either after the formation of the hydrogel or before administering the hydrogel, here the bacterial suspension was injected with the hydrogel by encapsulating it within its matrix. Here too, the QCS40P3–Odex showed significantly higher antibacterial activity as opposed to the QCS40-Odex, thus confirming the effectiveness of combining QCS with PANi. Also, the QCS-g-polyaniline/Odex hydrogel was found to be non-toxic to seeded C2C12 cells and cell proliferation increased with increasing PANi content in the hydrogel [123].

As mentioned previously, colloidal PANi particles in the form of aqueous dispersions have also been prepared. This is done by oxidising aniline in an aqueous medium and in the presence of water-soluble polymer as a steric stabiliser, which prevents it from precipitating into insoluble powder, which is difficult to process. Numerous studies have been carried out to create colloidal PANi suspensions using different polymers as stabilisers, such as cellulose ethers [124], poly(methyl vinyl ether) [125] poly(vinyl alcohol) [125], PEO [126] and poly(vinyl alcohol-covinyl acetate) [127]. A study performed to consider antibacterial effects of PANi suspensions was carried out by Kucekova *et al* [128]. Here, colloidal PANi suspension using poly(N-vinylpyrrolidone) stabiliser was prepared and assessed against *S. aureus, B. cereus, E. coli* and *P. aeruginosa.* The study revealed that the PANi suspension did possess antibacterial properties and was most pronounced for *B. cereus* and *E. coli*, with a minimum inhibitory concentration of 3500 g ml^{-1} [128]. A study carried out by Kašpárková *et al* [129] revealed that the antibacterial activity of colloids depends both on the type of stabilizer used and on the ratio between the stabilizer and PANi, with increasing PANi content demonstrating higher antibacterial capacity [129].

The recent approach by Tang *et al* [130] of using PANi for a photothermal antibacterial (PTA) strategy has been a major overhaul in tailoring the antibacterial mechanism of PANi (figure 11). They carried out the synthesis of PANi-poly (p-styrenesulfonic acid) (PSS) (PANi:PSS) with amphiphilic features, which allow it to translocate across the bacterial cell membrane without overt disruption or poration of the cell membrane, subsequently using its photothermal property to induce hypothermal damage to the intracellular content. This approach allows for higher killing efficiency as desired molecules can be selectively targeted for disruption. The strategy has also been gaining interest lately due to its higher efficacy in controlling the growth of multidrug-resistant bacteria and also in preventing the formation of a bacterial film. For the synthesis of PANi:PSS, biological system compatibility was important and therefore to achieve this, an enzyme-mimetic aqueous synthesis method was introduced using iron phosphates nanozyme as the catalyst and H_2O_2 as the initiator [78]. The water solubility of PANi:PSS was fundamental and selected as a macromolecular template to guide its synthesis using sulfonated polystyrene to promote the head-to-tail coupling of aniline monomers [130]. Using Calcein-AM/PI staining, the localization of as-prepared PANi:PSS was determined using confocal microscopy and the results confirmed its rapid entry into the bacterial cell without killing it (figure 12). Furthermore, the absence of any significant retention of the PANi:PSS on the cell membranes within 2 h, also indicated that the interactions between the PANi:PSS and the bacterial cell membrane did not originate from electrostatic activity. This is very important to minimize the side effects on normal mammalian cells caused by membrane disturbances involved in traditional

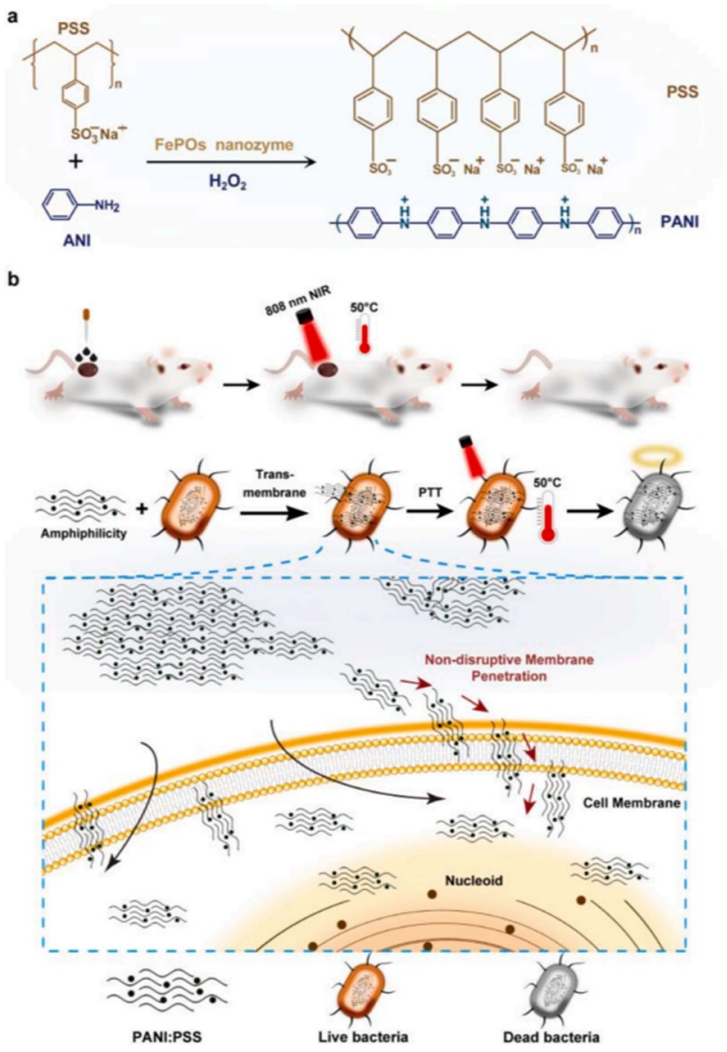

Figure 11. Schematic illustration of (a) the synthesis protocol of water-soluble PANi:PSS and (b) the spontaneous, non-disruptive translocation and photothermal antibacterial mechanism of water-soluble PANi:PSS against MRSA, *S. aureus*, and *E. Coli*. Reproduced with permission from [130]. CC BY-NC-ND 4.0.

positively charged antibacterial agents. When irradiated with NIR (808 nm) the internalised PANi:PSS was able to generate local hypothermia and destroyed *S. aureus*, Methicillin-resistant *Staphylococcus aureus* and *E. coli* efficiently as well as the corresponding biofilms (figure 12). Results from *in vivo* studies with water-soluble PANi:PSS using mice demonstrated excellent antibacterial activities without side effects. Therefore, the prepared water-soluble polymer can be considered to have great potential in the treatment of various bacterial infections.

As a result of its antibacterial properties, scientists have investigated PANi for various applications. Traditionally, numerous studies have been undertaken on developing antibacterial PANi conductive scaffolds for TE applications. In fact, recently, Wu *et al* [103] considered the use of EST of QCSP for chronic wound treatment. *In vivo* studies on diabetic rats showed that EST of QCSP was more effective in promoting healing of infected diabetic wounds than the conventional EST via rigid electrodes [103]. Studies on PANi based materials as antifouling coating have also been undertaken [131–134]. Kim *et al* [132] reported the development of a novel catechol-conjugated poly(vinylpyrrolidone) sulfobetaine (PVPS) and PANi tightly linked by ionic interaction (PVPS:PANi) as a photothermal antibacterial agent for surface coating. When stimulated with NIR light a sharp rise in the photothermal heat was triggered resulting in the rapid and effective killing of 99.9% of the Gram-positive and -negative bacteria tested within 3 min of NIR light exposure when used at the concentration of 1 mg ml^{-1} [132]. An electric field can induce non-specific bioadsorption on electrode surfaces, therefore minimising such biofouling on electrodynamic systems, Lee *et al* [133] looked into the use of self-doped sulfonated polyaniline (SPANI) coating as zwitterionic and conductive interfaces for anti-biofouling on open Au electrode surfaces. The zwitterionic material helps in the formation of a hydration layer through strong ionic salvation and thus helps in maintaining electrical

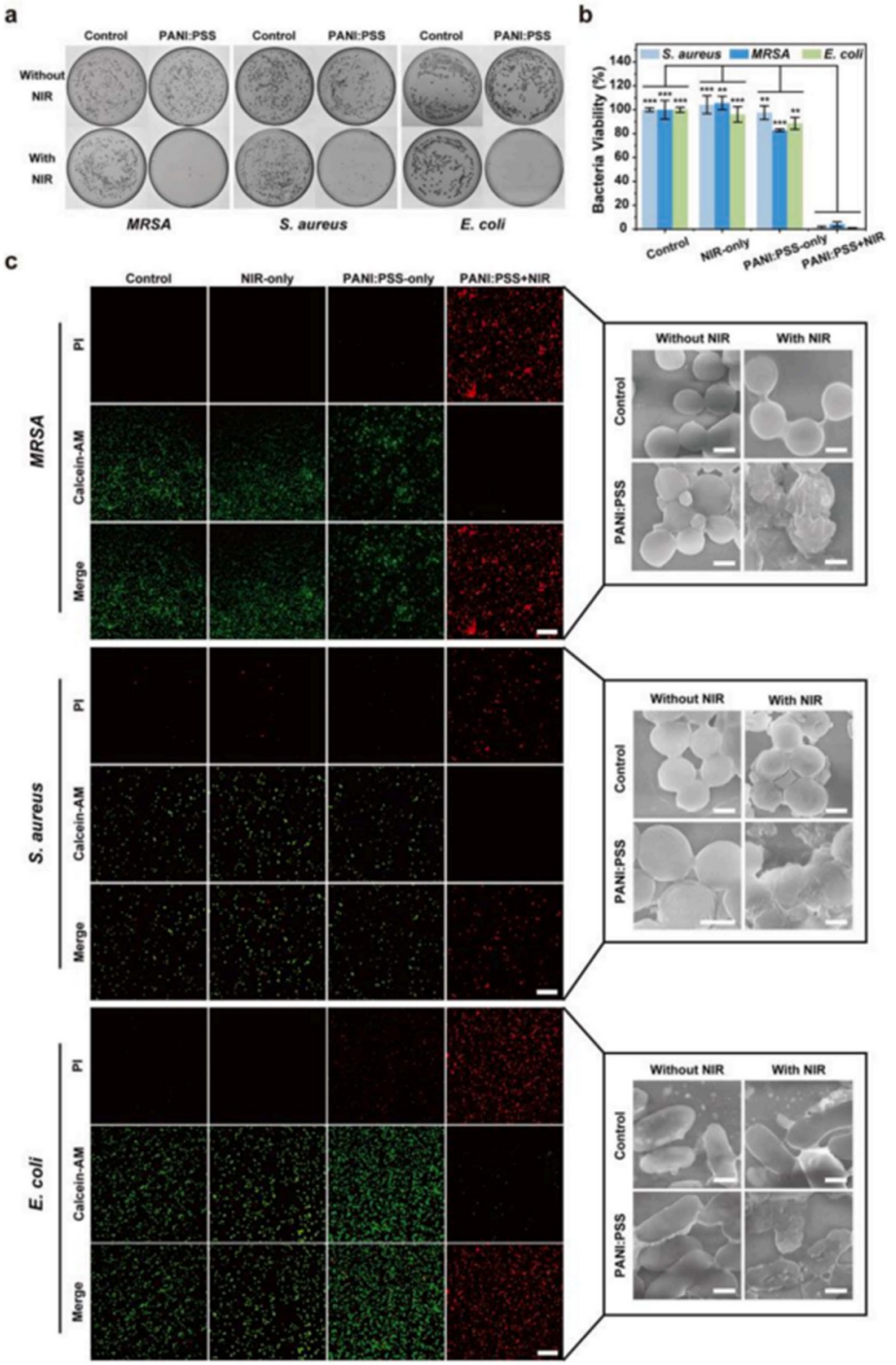

Figure 12. (a) Photographs of bacterial colonies and (b) viability data of MRSA, *S. aureus* and *E. coli* after coating the plate and incubating for 18 h. Data are expressed as mean ± standard deviation ($n = 3$). **represents $p < 0.01$ and ***represents $p < 0.001$. (c) Calcein-AM/PI dual staining fluorescence images captured by CLSM (red: PI, green: AM) and SEM images of MRSA, *S. aureus* and *E. coli*. Scale bars of CLSM images and SEM images represent 20 μm and 500 nm, respectively. NIR laser dose: 808 nm, 1.0 W cm^{-2}, 5 min. Reproduced with permission from [130]. CC BY-NC-ND 4.0.

neutrality thereby preventing electrostatic adsorption. The zwitterionic coating was developed by carrying out electrochemical polymerization of aniline on the Au electrode surface-functionalized with cysteamine ($HS–CH_2CH_2–NH_2$) followed by a post-polymerization treatment with fuming sulfuric acid. The SPANI-coated electrodes exhibited an excellent anti-biofouling ability, with a very low average residual mass rate of 1.44% in comparison to 14.3% for electrodes modified with poly(ethylene glycol) (PEG). In fact, even after 11 cycles of zwitterionic coating capturing and releasing/(dielectrophoresis (DEP)) process, SPANI had an average residual mass rate of 1.62% [133]. Conductive PANi has also been used for the preparation of antifouling ultrafiltration membranes. In the study carried out by Masim *et al* [135], the antibacterial, anti-corrosion and phosphate adsorbent capability of PANi-ZrO_2 composite was investigated for the first

time as a promising comprehensive treatment method for water filters in the aquaculture industry and for use in water purification applications. The antibacterial efficacy of the PANi-ZrO_2 composite was twice that of commercial ZrO_2 against *E. coli* and *S. aureus*. After 24 h of contact time, the highest phosphate removal efficiency for PANi-ZrO_2 was 64.4% whereas ZrO_2 and PANi exhibited removal efficiencies of 36.7% and 9.6%, respectively. Also, when coated on iron substrate and tested for anti-corrosion, no rust was formed. Similarly, a number of studies have been carried out for developing PANi based systems for better environmental remediation by providing efficient antimicrobial protection and detection and degradation of organic pollutants such as contaminating antibiotics via photolytic degradation [135]. In the study carried out by An *et al* [136], conductive PANi electrochemically deposited on an iron mesh-based metal-organic framework was able to successfully induce enhanced photolytic destruction of Thiamphenicol and inactivation of *E. coli* [136]. Another application has been the use of the antibacterial feature of PANi to develop antibacterial packaging materials. Youssef *et al* [137] developed paper sheet based on conductive PANi/PS/Ag-NPs nanocomposite and results from the antibacterial studies revealed that the PANi/PS/Ag-NPs nanocomposite treated paper sheets were effective against *S. aureus, P. aeruginosa,* and *C. Albicans* [137].

An acceleration of research/developmental efforts to amplify the antibacterial properties of PANi is taking place, which should lead to the expansion of its application not just for electroactive scaffolds for TE applications but also in other areas like drug delivery, photothermal therapy, coatings on medical prostheses and devices, and as antifouling ultrafiltration membranes, etc. In fact, the capability to infiltrate bacterial cells without harming them and then killing them on an induced trigger presents a real potential for using PANi for more targeted therapy approaches, thus further expanding its capability and scope of applications.

4. PANi in TE applications

4.1. Copolymer approaches

One major drawback limiting the processibility of PANi for various applications is its limited solubility due to the presence of a rigid backbone as seen with other π-conjugated polymers. Many studies have therefore been carried out to address this issue and one such strategy is to develop co-polymeric systems of polyaniline (co-PANi) which has been done by combining it with one or even more monomeric types for a cumulative benefit. Here again, different approaches for developing co-PANI have been undertaken, as discussed in this section.

4.1.1. Graft PANi copolymer

Graft PANi copolymers are segmented and generally consist of a linear backbone of one composition and randomly distributed branches of different compositions, with PANi being either the linear backbone or the branch and vice versa. Also, these graft-copolymers can be prepared either by (a) grafting through, (b) grafting from and (c) grafting to.

4.1.2. Block PANi copolymers

Block PANi copolymers consist of two or more covalently bonded monomers. These are prepared by controlled polymerisation of one monomer (aniline), followed by chain extension with a different monomer to form AB or ABS block copolymers.

4.1.3. Star- PANi copolymers

Star PANi copolymers consist of several linear polymer chains connected at one point. This can be further divided into two types: (a) A homo arm consisting of the same regular polymer chain which may also have a block monomeric arrangement, and (b) A miktoarm consisting of different heteroarms.

4.2. Applications of PANi co-polymer approaches

Studies undertaken on the copolymers of PANi demonstrate their increased solubility in a varying range of solvents both aqueous and non-aqueous based, over that of neat PANi that has limited solubility [138]. This increased solubility, which results in increased processibility, has further widened the scope for the application of the copolymer either on its own or in combination with other polymers and materials as blends or composites for different applications. Co-polymerising PANi has also positively impacted its two other critical parameters, namely biodegradability and biocompatibility. Neat PANi is non-biodegradable, however co-polymers of PANi and their subsequent processed forms neat, blends [138, 139] or as composites have been found to be biodegradable and biocompatible, which has further made it more attractive for a variety of applications, particularly as conductive scaffolds for TE applications, as discussed above.

A terpolymer composed of [PANi-co-(PDA-g-PLA)] was synthesized by Massoumi *et al* [109]. The authors chose to use PDA owing to its biocompatibility, good adhesive features and presence of chemical

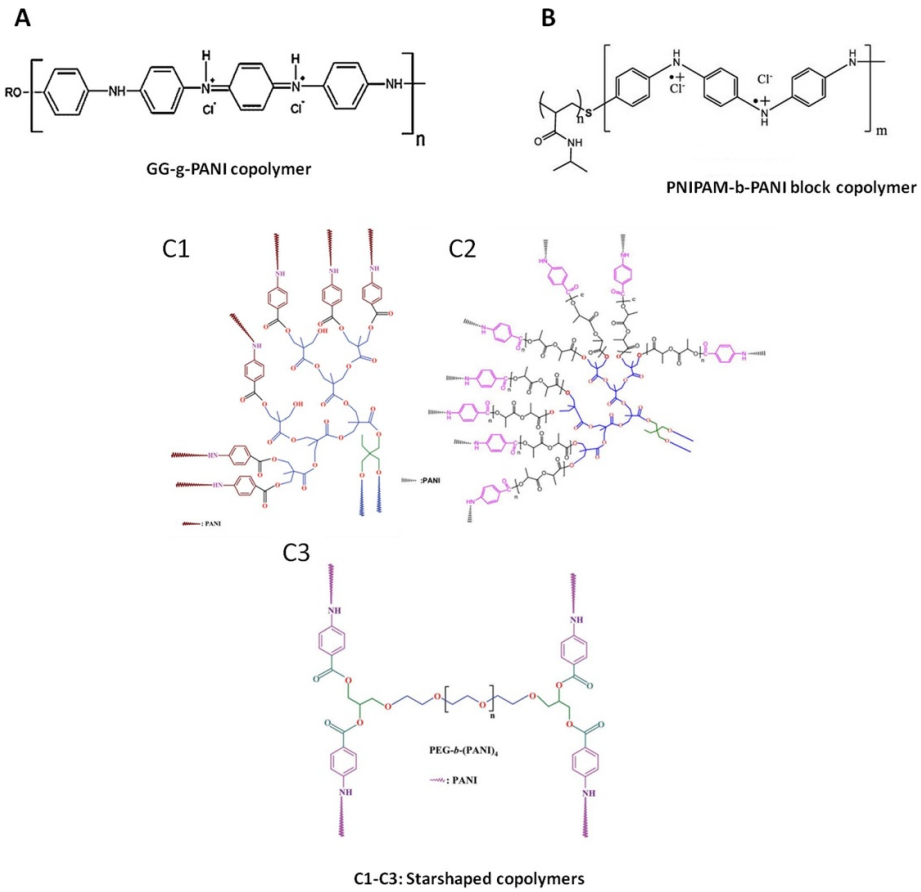

Figure 13. Various copolymers of PANi: (A) Gellan gum-graft-polyanilne copolymer (GG-g-PANi); (B) linear block copolymer containing a thermoresponsive block (poly(N-isopropylacrylamide), PNIPAM) and a near infrared (NIR) light-absorbing block (polyaniline, PANi), PNIPAM-b-PANi; (C1) Star-shaped polyaniline (S-PANi) with hyperbranched aliphatic polyester as the core; (C2) Star-shaped polyaniline (S-PANi) with star shaped hyperbranched aliphatic polyester (S-HAP) and poly(D,L-lactide) (PLA) as the core; (C3) H-shaped miktoarm PEGs-b-(PANi)4. (A) Reprinted from [142], Copyright (2015), with permission from Elsevier. (B) Reproduced from [143]. CC BY 4.0. (C1) Reproduced from [139] with permission from the Royal Society of Chemistry. (C2) [140] (29 December 2017), reprinted by permission of the publisher (Taylor & Francis Ltd, www.tandfonline.com.) (C3) Reproduced from [141] with permission from the Royal Society of Chemistry.

moieties that allows for post-modification. Similarly, PLA was used because of its inherent physicochemical as well as biological properties, like biocompatibility, bioresorbability and acceptable mechanical properties. Combining these two different polymers with PANi would result in the formation of a terpolymer that is conductive, bioadhesive, biocompatible and biodegradable, properties that are ideal as a scaffolding material for TE applications. The synthesis of the terpolymer was carried out using a one-step chemical oxidation polymerisation of aniline and dopamine monomers under acidic conditions to produce PANi-co-PDA. The lactide monomers were then grafted onto the PDA segment by ring-opening polymerisation. The resultant terpolymer was electrospun to produce a conductive porous nanofibrous scaffold and demonstrated excellent physicochemical properties (mechanical, conductivity, electroactivity, wettability, and morphology). *In vitro* biological studies with pre-osteoblast cells (MC3T3-E1) were carried out over a period of seven days. The cells showed good attachment, growth and proliferation on the terpolymer when compared to the control [109].

In recent years, a number of studies have been carried out on developing star-shaped based co-polymers of PANi as electroconductive matrices for TE applications (figure 13). These copolymers have atrracted interest because of their relatively low cost, highly functionalised well-defined structures and low crystallinity. Sarvari *et al* [139] developed a conductive fibrous scaffold based on star shaped PANi (S-PANi) blended with PCL. First, hyperbranched aliphatic polyesters (HAPs) (G3 and G6) were synthesized via a melt polycondensation reaction between tris(methylol)propan, and 2,2-bis(methylol)propionic acid. The HAPs synthesized were then further reacted with p-anthranilic acid to form the phenylamine-functionalized aliphatic hyperbranched polyester macromonomers (PhAG3M and PhAG6M). The synthesized macromonomers were subsequently used in both chemical and electrochemical oxidation copolymerizations with aniline monomer to produce two star-S-PANis with HAP cores. The S-PANi soluble in DMSO was then

processed into fibrous meshes by blending it with PCL. The fibrous scaffolds were found to possess a single-phase indicating good interaction with the incorporated PCL. This microstructural property of the scaffold along with its hydrophilic nature was conducive for the growth of the seeded mouse fibroblast L929 cells. After seven days of seeding, L929 cells showed better growth and proliferation on the fabricated scaffolds when compared with that of the negative control. Overall, this study demonstrated for the first time the potential of such star shaped graft copolymer of PANi for TE applications [139]. In another such study, Massoumi *et al* [140] developed a novel conductive and nanofibrous scaffold based on star-like hyperbranched terpolymers of aliphatic polyester–poly(D,L-lactide)–polyaniline (S-HAP–PLA–PANi) also for TE applications. Synthesis of the terpolymer involved firstly the reaction between the HAP with L-Lactide to obtain a star-shape hyperbranched PLA (S-HAP–PLA) copolymer. To this, aniline monomer was co-polymerised through chemical oxidation to produce S-HAP–PLA–PANi. This co-polymer of S-HAP–PLA–PANi again showed improved solubility in DMSO which helped in its processing into conductive nanofibrous scaffolds by blending it with PLA. The resulting S-HAP–PLA–PANi/PLA nanofibrous scaffold was found to be electroactive with a conductivity value of 0.05 S cm^{-1} and non-toxic when tested with mouse fibroblast L929 cells [140]. Hatamzadeh *et al* [141] carried out the synthesis of the H-shaped miktoarm PEGs-b-(PANi)4 (PEG2000-b-(PANi)4 and PEG6000-b-(PANi)4) and by combining them with PCL developed conductive nanofibrous scaffolds (PEG2000-b-(PANi)4/PCL and PEG6000-b-(PANi)4/PCL) for TE applications. The scaffolds were found to be hydrophilic and electrically conductive (PEG2000-b-(PANi)4/PCL: $\theta = 81 \pm 3.1°$, $\sigma = 0.003 \pm 2 \times 10^{-4}$ S cm^{-1}; PEG6000-b-(PANi)4/PCL: $\theta = 76 \pm 2.8°$, $\sigma = 0.0022 \pm 1.7 \times 10^{-4}$ S cm^{-1}). The cytocompatibility was assessed by growing mouse fibroblast L929 cells on the scaffolds. The number of cells growing on the scaffolds after seven days of culture was higher than that of the negative control [141]. All these studies firmly confirmed the synthesis of star-S-PANi copolymers and *in vitro* cell studies were very promising demonstrating their non-toxicity and desirability for TE applications. However, there was hardly any reporting on the *in vivo* effect of such polymer scaffolds. Therefore, it will be very insightful to understand the *in vivo* degradation and biocompatibility of these copolymers, for realistically considering them further in TE strategies.

An important advancement and broadening of the application of PANi has been the research centred on developing stimuli-responsive PANi based systems, in the form of films, scaffolds or more recently as hydrogels, for a range of biomedical applications, including TE, tailored cell therapy, drug delivery and photothermal therapy. Qu *et al* [144], developed a series of injectable conductive hydrogels as 'smart' drug carriers with the properties of electro-responsiveness and pH sensitivity. For the synthesis of the hydrogel, solutions of CP copolymer (4% w/v) and varying amounts of OD (1, 3 and 5% w/v) were mixed under physiological conditions to form the CP/OD hydrogels (CP/OD1, CP/OD3 and CP/OD5), respectively. All CP/OD hydrogels were conductive with conductivity values of 7.6×10^{-2} S m^{-1}, 7.8×10^{-2} S m^{-1}, and 7.9×10^{-2} S m^{-1} observed for CP/OD1, CP/OD3, and CP/OD5, respectively. Once the electroconductivity of the hydrogels was established, the authors also looked into the electrical stimuli response release capability of the incorporated drugs (amoxicillin and ibuprofen) from the hydrogel (figure 14). To do this, first, the cumulative release percentages of both amoxicillin and ibuprofen increased with increasing voltages, which was ascertained without any external stimulation. For amoxicillin, it was 69% and 82% at 1 and 3 V after 80 min as opposed to 34% via normal diffusion for the same time. A similar release pattern of the drug ibuprofen was observed when increasing voltage was applied. Moreover, the 'on-off' pulse triggered drug release capability of the gels was assessed by stimulating the gels with 3 V voltage for 3 min and repeated after every 30 min or 60 mins. For amoxicillin the release was 18%, 14% and 5% for the first, second and third stimulation, whereas for the ibuprofen the release was 5%, 4% and 2% for the first, second and third cycle, respectively. The higher percentage of release in the first cycle was due to the higher amount of loading in the hydrogels during the first cycle. Also, this higher release of the drugs when 'turned-on' (stimulated) as opposed to 'turned-off' (via normal diffusion) demonstrated the on-demand drug delivery potential of the hydrogel. Negatively charged drugs are released by reduction of CPs whereas positively charged drugs are released by oxidation [140], therefore the release of both the negatively charged amoxicillin and ibuprofen occurs under reducing conditions. As PANi gets reduced, the positive charge within the CP/OD hydrogels also reduces resulting in the expulsion of the drug molecules from the hydrogel. However, the material may contract, therefore, upon EST, the electric field also causes the movement of the drug molecules into electrodes bearing opposite charge, thereby avoiding contraction and release from the hydrogel. Similarly, the hydrogel also successfully demonstrated a pH-controlled response for drug release. When studied under two different pH conditions, an acidic environment (pH = 5.5) and a physiological environment (pH = 7.4), the hydrogel exhibited different release behaviour. At pH 7.4, only 23% of the drug was released after 45 min and the total cumulative release percentage of the hydrogel was about 55% for 36 h. Whereas in pH 5.5, approximately 55% of the drug was released after 45 min of incubation during the initial burst release period, and approximately 99% of the drug was released in the whole period of 36 h. These results clearly

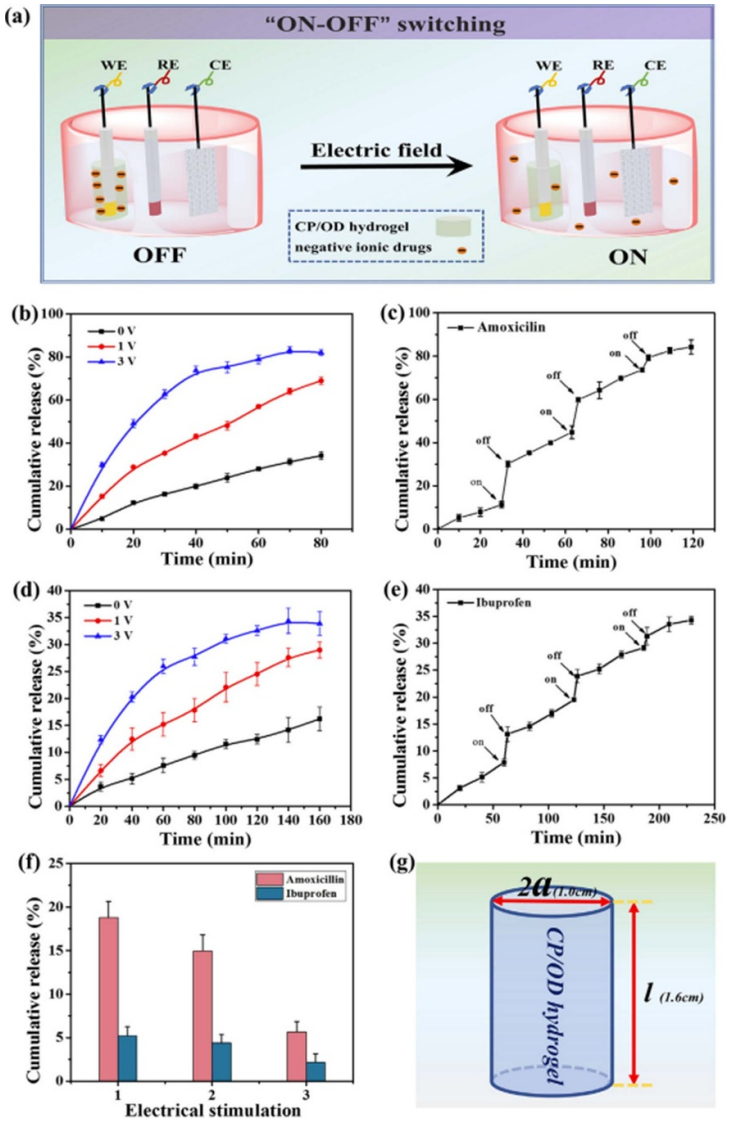

Figure 14. (a) Schematic illustration of pulse release of drug model from CP/OD conductive hydrogel. (b) Drug release study of amoxicillin in PBS with pH 7.4 under different electric potentials. (c) Drug release study of amoxicillin in PBS with pH 7.4 under an electric potential of 3 V for 3 min, repeated every 60 min. (d) Drug release study of ibuprofen in PBS with pH 7.4 under different electric potentials. (e) Drug release study of ibuprofen in PBS with pH 7.4 under an electric potential of 3 V for 3 min, repeated every 60 min. (f) Release ratio of amoxicillin and ibuprofen in PBS (pH 7.4) following an applied voltage (3 V) duration of 3 min, repeated every 30 min (amoxicillin) or 60 min (ibuprofen). (g) The parameters of drug-loaded CP/OD hydrogels. Reprinted from [144], Copyright (2018), with permission from Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

showed how pH can be used for tailoring the release of drugs from the hydrogel. In fact, the gelation time, mechanical properties and the *in vitro* degradation rate, all could be tailored by changing the concentration of the OD solution. The hydrogels were also found to be cytocompatible which was assessed by their direct interaction with L929 cells. Within day 1 and day 3 of seeding on the hydrogels, an increase proliferation of the cells was obtained, although no differences were observed between the samples (CP/OD), control (C/OD) and the TCP. However, on day 5 the growth on the samples was significantly higher than those on the C/OD control and the TCP. The hydrogel also exhibited inherent antibacterial properties due to the PANi component, exhibiting a 100% kill ratio for both *E. coli* and *S. aureus*. *In vivo* studies in Spring Dawley rats after seven days of implantation revealed mild inflammatory response. However, after 35 days the inflammation had reduced along with a significant reduction in the number of mast cells compared to that of day 7. Thus overall, the inherent antibacterial, non-toxic, biocompatible and stimuli responsive hydrogel exhibited great potential as a smart drug delivery vehicle for less invasive and more controlled precision release of drugs [144].

Hydrogels normally exhibit weak mechanical properties, lacking in strength for applications in tissues such as bones, cartilage and muscle, therefore, in another study (Li *et al* [145]) interpenetrating copolymeric

hydrogels based on gelatin-graft-PANi and carboxymethyl-CS crosslinked with oxidised dextran were synthesized to develop injectable hydrogels with enhanced mechanical properties [145]. With increase in the PANi content, the mechanical strength of the hydrogel had reduced but overall the storage modulus (571 kPa) was found to be higher in comparison to the PLA-PEG-PLA AT complex hydrogels, which had shown the highest modulus of about 1 kPa. Adipose derived mesenchymal stem cells and C2C12 myoblasts were used for testing the cytotoxicity of the hydrogels via indirect testing by culturing the cells on hydrogel extracted solution prepared by incubating the hydrogels in the culture medium for 24 h and 30 days at 37 °C. Results from LIVE/DEAD assay showed that after 24 h, both adipose derived mesenchymal stem cells (ADMSC) and C2C12 exhibited a normal cell morphology with few dead cells demonstrating its non-toxicity. When assessed for *in vivo* biocompatibility by subcutaneous injection in Sprague Dawley rats for one and four weeks, little fibrous capsule was found around all the hydrogels. Also, an increase in the inflammatory cells was found after four weeks when compared to week 1, which the authors attributed to the hydrogel undergoing degradation [145].

Exploration of PANi copolymer for photothermal therapy has also been undertaken. Abel *et al* [143] carried out the copolymerisation of PANi as a near infrared (NIR) light-absorbing block with poly(N-isopropylacrylamide) (PNIPAM) a thermoresponsive block to develop a linear block copolymer of PNIPAM-b-PANI. For the synthesis, PNIPAM polymer terminated with aniline moiety (ANI) was first synthesized using a radical vinyl polymerisation. In the second step using oxidative polymerisation, PANI block is synthesised, with its synthesis initiated at the ANI group present in the PNIPAM to link its chains with PANI. The chemical structural organisation of the block PNIPAM-b-PANI co-polymer was established using proton nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FT-IR). The copolymer was found to be soluble in a range of solvents: aqueous (water $= 8.3\ \mathrm{g\,l^{-1}}$) and non–aqueous solvents such as ethanol, formic acid and acetonitrile and many more as opposed to pure PANi that has limited solubility. Importantly, the copolymer was thermally responsive due to the PNIPAM component with a lower critical solution temperature (LCST) at 34 °C and also conductive with a resistivity of $5.3 \times 10^{-4}\ \Omega$ cm due to the presence of the conductive PANi block. When irradiated with NIR light (785 nm, 100 mW) the PANi block is able to absorb the light and heat up, consequently triggering a phase transition in the PNIPAM block ultimately leading to its morphological transition from a coil (extended) to globular (folded) shaped aggregates as observed on the surface of the PNIPAM-b-PANi film using turbidimetry and AFM. This ability of the copolymer to undergo morphological transition when exposed to NIR light highlights its capability as a photothemal therapy agent as NIR can penetrate up to several centimetres in biological tissues [143].

Electromagnetic materials have wide ranging applications, therefore studies have also been carried out to develop such materials based on PANi. A novel conductive PANi-g-polystrene/FeO_4 nanocomposite with both electrical and magnetic properties was developed by Ahmadkhani *et al* [146] for the treatment of cancer. The authors used a novel approach combining atom transfer polymerization (ATRP), a type of controlled/living radical polymerization (CRP), which results in well-defined polymers because of controlled molecular weight, narrow molecular weight distribution, combination and performance [146, 147] and *in situ* chemical oxidation of aniline from the surface of the magnetic NP to form the final product. More specifically, first, the ATRP synthesis of styrene using an ATRP initiator attached to the surface of Fe_3O_4 NPs, followed by functionalization of the Fe_3O_4-PSt with amine groups ($–NH_2$) was carried out. Secondly, *in situ* graft copolymerisation of aniline via chemical oxidation on the amine groups site of Fe_3O_4-PSt was performed, leading to the final product with Fe_3O_4 as the core and a PSt-g-PANi as a shell. FT-IR, X-ray diffraction (XRD), thermogravimetric analysis (TGA), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) confirmed the success of the synthesis and the polymerisation processes. The Fe_3O_4/PSt-g-PANi particle was found to have a particle size of 12–50 nm and shell thickness of 7–14 nm. The synthesized Fe_3O_4/polystyrene-g-PANI also had better electrical conductivity and thermal stability when compared to the homopolymers. This approach, therefore, highlighted a more systemic method for developing these conductive and magnetic nanocomposites over the traditional approach which involves just mixing two different polymer components. Although this study is very informative on the development of such PANi based conductive and magnetic material, it will also be insightful to understand the degradation and biocompatibility of the material, which was not reported in this study [146].

To summarise, various synthetic reaction designs have been explored in the past few years to develop copolymers of PANi that are biodegradable. The synthesis has also progressed from traditional, grafting, block polymer synthesis to incorporating star based and other CRP polymerisation to even form metal/organic co-polymeric composite systems that are biodegradable. These co-polymers of PANi apart from being used as typical electroactive matrices for TE applications are also explored for tailored applications of controlled cell therapy, drug delivery, electromagnetic and photothermal therapy.

4.3. Blends and composites incorporating PANi

As mentioned previously, in the last decade, numerous studies have been carried out using scaffolds containing PANi in combination with bioresorbable polymers for a range of TE applications, including cardiac, nerve, muscle, bone, skin, i.e. wound healing, and others. In some of the studies, either a natural or a synthetic polymer was used, or combinations of both, such as PCL (Song *et al* [148]; Li *et al* [149], Garrudo *et al* [150]), PLA (Eslami-Arshaghi *et al* [151]; Wang *et al* [152]; Chen *et al* [153]), CS (Mawad *et al* [98]; Badhe *et al* [11]; Daraeinejad and Shabani [81]), gelatin (Wang *et al* [154]), P3HB (Pramanik *et al* [155]) and collagen in combinations with other natural polymers (Roshanbinfar *et al* [19]; Arteshi *et al* [156]). In all of the published studies, which tested the PANi-based scaffolds by means of *in-vitro* cell culture methods, the authors reported that the addition of PANi had a beneficial effect on the overall cell attachment and proliferation behaviour.

In comparison to the numerous *in-vitro* cell studies that have been carried out, very few *in vivo* studies have been performed using such blends and composites of PANi. Badhe *et al* [11] investigated a CS based hydrogel scaffold incorporating vitamin D in an animal model using Wistar albino rats. The animals were put into four groups, i.e. no treatment, treatment with cipladine, blank hydrogel and CP-based hydrogel. The results showed that the CP-based hydrogel accelerated wound healing significantly compared to the commercial treatment and hydrogel alone. The histopathology analysis revealed that collagen synthesis, fibroblast migration and epithelization could be seen in the hydrogel groups. The animals in group 2 (commercial treatment) showed necrosis, indicating scar formation.

In another study, Sun *et al* [73] developed a multifunctional doping acid crosslinking technique to gelate conductive polymer hydrogels using phytic acid (IP6). Electrospun PCL based scaffolds, which had been coated with a PANi hydrogel in an *in vivo* model in Sprague-Dawley rats, were investigated. Because phytic acid possesses ion conducting properties, the coated scaffolds showed a relatively high conductivity of 0.11 S cm^{-1}. The coated and uncoated scaffolds were implanted into the backs of the rats, two scaffolds of each type in each rat. The samples were extracted after one, two and four weeks after surgery. No behavioural changes or physical impairment were observed, which could indicate systemic or neurological toxicity. However, both types of scaffolds, the PANi coated PCL scaffolds and the blank PCL scaffold, were found to be surrounded by fibrous tissue. Mild to moderate histological response could be observed, which decreased to mild after four weeks for both types of scaffolds. The PANi coated scaffold showed a better histological response overall. The coated scaffold exhibited a moderate response after seven days of implantation characterized by the moderate infiltration of lymphocytes and the formation of multinucleated giant cells and hyperplasia of fibrous tissue. Overall, the *in vivo* studies suggested that the IP6-gelated PANi hydrogel had superior *in vivo* biocompatibility compared to the PCL scaffold [73].

4.4. Composite hydrogels comprising a bioactive or ceramic phase

A wide range of hydrogels comprising inherently conductive polymers such as PANi are being investigated for applications ranging from bone to cardiac TE, the addition of filler particles, in particular conductive fillers, is gaining in importance. However, there is also an increasing number of scientific publications using bioactive fillers to confer the matrix with a bioactive property to optimise cell attachment and proliferation. One example of such a nanocomposite, composed of CS-gelatin, nanohydroxyapatite and PANi, was synthesised using a straightforward blending method followed by lyophilisation [157]. The first step involved the preparation of a 2% w/v CS solution in aqueous acetic acid (1% v/v), which was stirred until the CS had dissolved completely. The second step involved the preparation of the solution of gelatin in water at 40 °C, to which a given amount of nHA was added. Subsequently, the gelatin and CS solutions were mixed together and PANi was added before the addition of glutaraldehyde. The solution was then crosslinked and later lyophilized twice at −80 °C for 48 h. The scaffolds were highly porous with a mean pore size of approximately 200 μm. The tensile and compressive strengths of the scaffolds were clearly improved by more than 100% and around 50%, respectively, compared to the unreinforced hydrogels without nanofillers and without PANi. The bioactivity of the composite scaffolds containing nHA and PANi-nHA was evaluated using SBF studies, which showed that the composite scaffolds showed excellent mineralization potential as shown by the formation of apatite on their surfaces. In particular the presence of both PANi and nHA led to increased mineral deposition. The authors suggested TE of hard tissues as a possible application area [157].

A recent study reported on a conductive composite hydrogel/fibre scaffold, which contains a conductive phase both in the matrix in the form of graphene NPs and in the fibrous phase in the form of PANIi [158]. For the preparation of this composite, PANi based fibres were first fabricated using electrospinning and were then transformed into a three-dimensional structure using ultrasonication. The ultrasonication step was performed with an intensity of 15 W cm^{-2}, a duty-cycle of 50% and a duration of 7.5 min. The 3D fibrous structure thus synthesized was then mixed with the hydrogel precursor solution consisting of predetermined fractions of oxidized polysaccharides, gelatin and graphene and then left to gel. The composites containing

fibres showed an increase in Young's modulus, roughness as well as electrical conductivity with a decrease in hydrophilicity. Osteoblast-like cells showed a significantly increased activity compared to the PCL hydrogel alone, in particular after three and seven days in culture.

BC aerogel possesses a highly porous 3D structure, which provides an ideal matrix for embedding nanomaterials and polymers. In a recent study, a BC based aerogel was investigated as the matrix for an electrically conductive composite for TE [159]. The BC composites with the addition of nano-clay and PANi were synthesized using a two-step procedure. Initially, clay nanoplatelets were dispersed in the BC membrane thus forming a nanofibrillated template for aniline *in-situ* polymerization, which led to the formation of a double interconnected network of an electrically conductive paths within the matrix. A surface electrical conductivity of 0.49 S cm^{-1} was measured for composite aerogels with 5 wt% nano-clay, which was 16 times higher than for BC aerogels without nano-clay. Thermal stability and storage modulus of the aerogels was improved by inclusion of PANi and nano-clay. Cell adhesion and biocompatibility were improved in the composites (together with a synergistic effect between the nano-clay and PANi inclusions) as compared to the unreinforced samples. No mutagenic or carcinogenic effects were found in the study. The electrically conductive composite aerogel scaffolds could be applied for TE applications [159].

Hosseini *et al* [15] also developed a lightweight aerogel comprising PANi, silver NPs and BC with the proposed application as a scaffold for soft TE. Cellulose based aerogels are currently gaining increasing significance due to their improved properties compared to mineral based aerogels. The aerogels were synthesized using BC hydrogels and varying concentrations of silver nitrate ($AgNO_3$) ranging from 1 to 5 mg. The fabrication process involved mixing of PEG and aniline monomers in a mixture of water and HCl (using two different molar ratios), before the addition of the BC. Subsequently, the mixture was immersed into a predetermined concentration of $AgNO_3$ solution. The mixtures were subjected to freeze-drying to obtain the finished aerogels. The samples were then studied in terms of cell viability using mouse embryonic fibroblasts (L929 cells) for up to seven days. It was reported that the cell proliferation in the ternary nanocomposite aerogels dramatically increased [15].

Inherently conductive particles such as graphene (G), GO and other NPs hold great potential to improve the electroconductive properties of conductive polymer matrices. In a study from 2016, Mahmoudifard *et al* [160] investigated the incorporation of graphene and GO nanosheets into a matrix of PAN/PANi-CSA, which was then electrospun into a nanofibrous scaffold using DMF as a solvent, and could show that the incorporation of the fillers increased the biocompatibility as well as the electrical conductivity significantly. The cultured cells on composite nanofibrous PAN/PANi-CSA/G confirmed a higher proliferation and differentiation value compared to other groups including PAN/PANi-CSA/GO and PAN/PANi-CSA scaffolds. The study used muscle satellite cells enriched by a pre-plating technique and studied cell spreading and proliferation, which were found to be the highest for PAN/PANi-CSA/G. The authors hypothesized that the high conductivity in combination with the relatively higher stiffness of the PAN/PANi-CSA/G composite nanofibers lead to an enhancement of proliferation and differentiation of satellite cells [160].

A scaffold for the treatment of peripheral nerve damage, where high values for conductivity are of extreme importance, was developed using silk, reduced graphene oxide, and PANi, obtaining a highly conductive silk knitted composite [161]. The composite scaffold was prepared by two-step electrostatic self-assembly and the composition of the individual components was varied to optimise both conductive and mechanical properties, with ultimate strength, elongation at break, and elastic modulus of ∼30 MPa, ∼107%, and 11 MPa on average, respectively. The conductive scaffolds had ordered loops, fibre structure, and large pore sizes between 40 and 70 μm. The conductivity of the scaffolds ranged between 0.62×10^{-3} and 1.72×10^{-3} S cm^{-1}, which was proposed to be adequate for nerve TE applications [161].

MWCNTs were incorporated into PANi and the thermoresponsive polymer PNIPAm to form a hybrid stimuli-responsive nanocomposite for applications in wound healing [162]. The PANi-MWCNT/PNIPAm composite scaffolds were fabricated using *in-situ* polymerization and subsequent electrospinning. The authors demonstrated physiological temperature coordinated cell grafting phenomena (using fibroblast cells) on the surface of the electrospun scaffolds. The principle behind the developed scaffolds lies in the encapsulation of cells inside the structure. The proposed *in situ* formation of the cell/scaffold construct facilitates the straightforward delivery of relevant cells, nutrients and growth factors to the wound site. The construct composed of scaffold, cells and growth factors can be incubated at 37 °C (above the LCST), whereby a gel is formed, which provides an ideal environment for cell proliferation. Thus, an inflammation-sensitive, stimuli-responsive scaffold has been manufactured [162]. A cell proliferation study was conducted as part of this research work, showing that fibroblasts could attach and proliferate well on the composite scaffold (significantly more than on PNIPAm and PANi/CNT), but at a temperature above LCST, i.e. 37 °C, cell proliferation was even greater. The authors of the study attributed this effect to the activity of the hydrophilic moieties of the individual materials in the composite, i.e. N–H, COOH, CONH, which become active and induce hydrophilicity in the composite at temperatures below the LCST. The increased

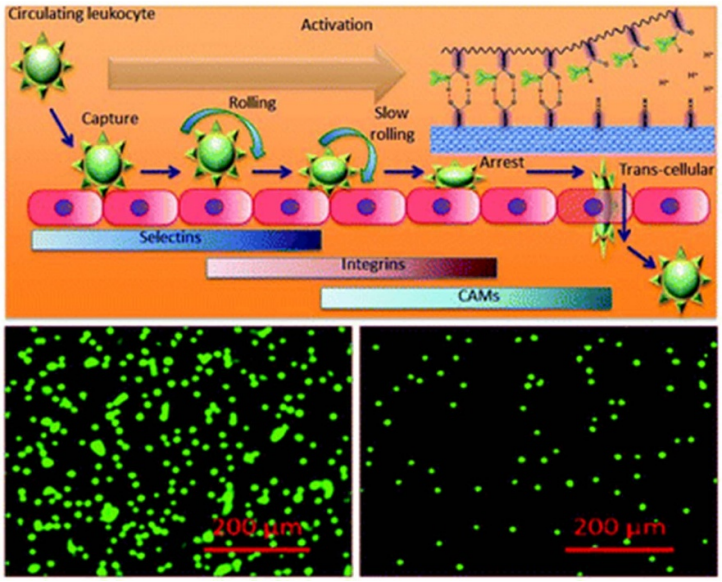

Figure 15. Schematic illustrating inflammation and response to the composite developed in [162]: (top) the keys and regulators in inflammation *in vivo* and the dynamics of leukocyte transmigration (bottom). The real time transmigrated leukocytes in the absence (left panel) and presence of the composite (right panel). Reproduced from [162] with permission from the Royal Society of Chemistry.

hydrophilicity results in the cells interacting less with the medium and their cytoskeleton structure becomes reorganized, so that they are less proliferative on the surface. When the temperature reaches or rises above the LCST, the hydrophobic moieties (isopropyl groups) become dominant, which leads to a scaffold on which cells can grow and proliferate.

The study furthermore investigated the inflammation process, which occurs during one of the stages of wound healing. During this process, inflammation associated loco-regional hypoxia, low pH and leukocyte extravasation into the perivascular compartment play a major role. The latter is a multi-step process organized by sequential activation-dependent cross talk between endothelial cells and leukocytes. The composite scaffolds were investigated for their inflammation sensitivity by the modified leukocyte transmigration assay. Leukocytes, stained using LeukoTracker™, are allowed for extravasation through the HUVEC in the presence (+) and absence (−) of tumor necrosis factor-alpha (TNFα) with respect to the control (without leukocyte). The transmigrated leukocytes were measured through relative fluorescence. The authors determined that the inflammation sensitivity decreased by about 50% compared to the control [162].

The upper panel of figure 15 [162] shows a schematic diagram of inflammation process, for which leukocyte interaction with vascular endothelium is required, and consists of several successive steps including circulating leukocyte capture, rolling, arrest, firm adhesion and subsequent transmigration. Fluorescence microscopy images of real time leukocyte extravasation without composite (left panel) and with composite (right panel) are shown.

In another study, zinc silicate (Willemite) NPs were sprayed onto a matrix composed of PANi, gelatin and PCL fibres, which had been fabricated using the electrospinning method, using plasma spraying in order to fabricate conductive nanofibrous composite scaffolds for bone TE applications. Interestingly, cell proliferation could be greatly enhanced on the fibrous-NP construct compared to both NP and fibrous scaffold alone. Moreover, common bone markers, such as alkaline phosphatase, alizarin red test and calcium salt precipitation, were increased significantly, suggesting increased differentiation of bone tissue [10].

Another approach altogether was followed in a recent study, in which electrospinning was used to fabricate nanoscaled PCL fibres, which were loaded with PANi coated titanium oxide NPs (TiO_2/PANI), which had been produced via oxidative chemical polymerization. The TiO_2/PANi composite NPs were mixed with PCL in varying concentrations (1–5 wt.%) and electrospun in a chloroform/methanol solvent system [21].

Some of the samples investigated also contained a model drug, namely simvastatin, which is used as a cholesterol reducing agent and as a bone regeneration drug in patients with osteoporosis. The results indicate that with increasing amount of TiO_2/PANi NPs the rate of drug release rose, additionally the composite NPs led to a higher hydrophilicity of the electrospun fibres. Cell proliferation studies with MC3T3-E1 osteoblastic

mouse cell line indicated that there was a statistically significant increase in cell proliferation after several days in culture. The electroconductive potential of the composite scaffolds was not evaluated in this study [21].

Titanium oxide nanotubes were also coated with PANi as a potential scaffold for TE of hard tissues [163]. The authors of the study combined CS-cross-linked PANi nanonets with titanium oxide nanotubes (TiO_2NTs). The composites, which were both osteoconductive and osteoinductive, were fabricated using electrochemical anodization followed by annealing at 430 °C and subsequent potentiostatic electropolymerization by cyclic voltammetric technique to compensate for the drawbacks associated with titanium oxide alone. The biocomposites showed strong anticorrosion properties, bioactivity *in vitro*, and good biocompatibility in cell culture with human bone marrow-derived mesenchymal stem cells (hBM-MSCs), whereby cell proliferation was significantly greater compared to TiO_2NTs without PANi. This was also shown by the enhanced expression of bone-related genes, including collagen-I, OPN, OCN, and RUNX 2, which was studied over a period of 14 days [163].

In another recent study, titanium oxide nanotubes (TNTs) were coated with PANi and subsequently used as a filler in PCL nanofibers [164]. The TNTs were fabricated using an electrochemical anodization process. The second step involved potentiostatic electropolymerization of aniline monomer to achieve TNT coated PANi substrate using cyclic voltammetry method at low temperatures. Prior to the coating process, the crystallinity of TNTs was achieved by an annealing step at 420 °C for two hours. The PANi coating forms a sheath around the nanotubes thus protecting the substrate from corrosion. The large surface area to volume ratio of TNTs led to improved properties in biocompatibility, electrical conductivity, hydrophilicity and biomineralization after coating with PANI. The attachment and proliferation of preosteoblast (MC3T3-E1) cells was found to be significantly higher compared to uncoated nanotubes and was proposed for bone TE. Osteogenic markers (such as ALP level, collagen type I secretion) were also analysed. This type of surface modification thus tailoring TNTs present an interesting approach for bone TE [21].

Most of the PANi based TE scaffolds use camphorsulfonic acid (CSA) as a dopant [19, 32, 156, 160]. The use of CSA as a dopant is widespread for PANI-based materials as it is able to preserve the conductivity of PANi in a broad pH range. However, there are some reports in the literature indicating that the detachment of CSA from PANi *in vivo* may lead to a reduction in cell proliferation and CSA has been shown to be moderately toxic in cell culture studies [81]. Therefore, alternative dopants are being investigated by a number of different research groups [63, 84]. Almasi *et al* [84] used GO nanosheets, which possess long pairs of electrons on their oxygen atoms as a dopant. GO NPs at a ratio of 0.06% were used for doping PANi, and subsequently the doped PANi was combined with PAN and electrospun at a ratio of 2.4:1 in DMF. The scaffolds were then studied in terms of microstructure and *in-vitro* cell viability with cardiomyocytes. It was found that the scaffolds comprising GO NPs led to a significantly higher degree of cell proliferation as well as the expression of cardiac related gene markers, compared to PAN-PANi scaffolds without GO NPs. The corresponding particles were embedded in PANi scaffold at a biocompatible concentration, and the composite scaffold of PANi-NPs was modified using plasma treatment to obtain a hydrophilic surface for cell attachment. GO NPs kept the interaction with PANi in physiological pH and hence, the cell proliferation on the composite scaffold showed the highest value of biocompatibility compared to the sample without NPs and the control group. FTIR spectroscopy studies confirmed the electrostatic/hydrogen bonds between GO nanosheets and PANi. The better cell adhesion, higher gene expression and stronger positive result of protein markers with the composite scaffold exposed the efficient interactions of GO NPs with PANi over cell culture.

To summarize, there have been many interesting research studies combining PANi with other conductive or bioactive particles, in which mostly it could be shown that the cell behaviour could be enhanced greatly. However, the difficulty of such multiphase systems for applications in TE is that the degradation *in vivo* is complex and it is thus difficult to ensure the safety of such a biomedical device, as long-term *in vivo* studies would be required.

In recent years, AOs in the form of AD, ATs, TA and AP have also attracted research interest. This is mainly because AOs are biodegradable unlike their larger molecular weight counterpart PANi which is non biodegradable in its pure form. As this paper is focused on PANi, applications of AO in TE will not be covered. All discussed studies on the applications of PANi in various TE approaches have highlighted the potential of PANi as a truly electroactive material for tissue regeneration. Table 1 shows a summary of selected successful studies on the application of PANi in different TE strategies, giving information on the dopant used, the achieved conductivity values and scaffold fabrication techniques. Tissues considered are bone, cardiac tissue, nerve and skin.

The scope of applications in TE further expands as new processing advancements and modifications continue to take place to enhance the various properties of these matrices such as solubility, processibility, antibacterial potential, biodegradability and biocompatibility. In fact, the concerns surrounding the non biodegradability of pure PANi and potential carcinogenicity issues have always been a contentious point when considering TE application of PANi. Therefore, as more research is carried out, more relevant data is

Table 1. Selected studies on PANi based scaffolds in tissue engineering.

System	Dopant	Conductivity/ measurement	Fabrication method	Application	References
PANi/PCL	CSA	10 μS/Two probe	Electrospinning/ hydrogel synthesis	Bone	Khorshidi *et al* [158]
PANi/chit	Phytic acid	0.16 S cm^{-1} Cyclic voltammetry	Film casting	Cardiac TE	Mawad *et al* [98]
PANi/PAN	CSA	0.38 S cm^{-1}/ Two-probe	Electrospinning in DMF	Nerve TE	Hosseinzadeh *et al* [79]
PANi/Chit	CSA	2.6×10^{-5} S m^{-1} Four-probe technique	Electrospinning in TFA/DCM	Wound healing	Moutsatsou *et al* [12]
PANi-grafted chitosan, Ag NPs	APS	/	Copolymerisation	Tissue engineering in general	Sultana *et al* [165]
PANi/RGO/silk knitted scaffold	RGO	1.7×10^{-3} S cm^{-1}/ Two-probe	Impregnation-drying-reduction method and *in-situ* polymerisation	Peripheral nerve TE	Meng *et al* [161]
PANi/GO nanosheets	GO	No value reported/not measured	Electrospinning followed by plasma treatment	Cardiac TE	Almasi *et al* [84]
PANi/bacterial cellulose/Ag NPs aerogels	APS	No values reported/ Closed-circuit test	Hydrogel synthesis and freeze drying	Soft TE	Pournaqi *et al* [18]
PANi coated TiO_2 NP-PCL nanofibers	APS	No value reported/not measured	Electrospinning	Bone TE	Rezk *et al* [21]
PANi/collagen/ hyaluronic acid	CSA	10^{-4} up to 300 S cm^{-1}/ Four-point technique	Electrospinning	Cardiac TE	Roshanbinfar *et al* [19]
PANi/PLA/PEO	CSA	80 S cm^{-1}/ Two-probe	Electrospinning	General TE	Munawar *et al* [32]
SiO_2 Fe_2O_3/PANi nanocomposites	APS	1.1 S cm^{-1}/ Electric resistivity measurement	Sol-gel synthesis	Bone TE	Lalegül and Elçin [166]
PANi/MWCNT/ PNIPAm	MWCNT	No value reported/not measured	*In-situ* polymerization/ electrospinning	Wound healing and other applications	Patra *et al* [162]
PCL/PANi	HCL	2.8×10^{-4} S cm^{-1}/ four point probe	3-D printing	Bone TE	Wibowo *et al* [74]

generated, particularly *in vivo*, and thus more confidence will grow on PANi suitability as a potential candidate for engineering excitable tissues as well as for wound healing.

5. Concluding remarks and outlook

In the past three decades PANi has been able to establish itself as one of the materials of choice among the conductive polymers. The relatively easy synthesis, stability and conductive nature of PANi are attractive properties that first made this polymer interesting for several applications. The fact that conductive polymers like PANi could provide essential electrical cues with or without EST for regenerating electrically excitable tissues like nerve and muscle, was a pivotal finding two decades ago, which further cemented research into PANi. Since then, PANi has been continuously explored for TE applications, which have been highlighted in this review, along with all the latest developments taking place in various connected important aspects such as biocompatibility, biodegradability, processing and antibacterial properties.

Doping remains a critical step in influencing the conductivity of PANi, and in recent years numerous research efforts have centred on exploring less toxic, bioactive and even natural biological molecules as dopants, which can also be integrated with PANi to make it self-doping, thereby imparting PANi the ability to maintain its conductivity for longer periods and even under physiological conditions. Another huge

ongoing effort is the development of biodegradable PANi and PANi based matrices. The fact that PANi in all its oxidation states is non biodegradable has always been a major concern for TE applications. The shift from fabricating simple blends and composites of PANi to synthesising copolymers containing biodegradable moieties amenable to enzymatic degradation and hydrolysis has taken a centre stage. A lot of these studies involve using oligomers such as AT, TA and AP. These PANi copolymers in addition to being biodegradable are also found to be biocompatible, hence further strengthening the efficacy of PANi for biomedical applications. In the past few years processing of PANi has also advanced, with more insight gained on the effect of reactant materials, the type of dopant used, and processing steps such as repeated purification, on the morphology of the final fabricated PANi scaffold and on its interaction with cells, determining its final outcome and application. In fact, because of the increased understanding and advanced processing of biocompatible PANi, it is now possible to incorporate living cells within PANi based hydrogels for bioprinting. This further opens a whole new avenue for utilising PANi for TE applications. Another crucial favourable characteristic of PANi is its antibacterial property, which again makes PANi based scaffolds ideal choice for biomedical applications particularly, as such scaffolds can fight possible microbial infection whilst providing a support matrix for the cells to adhere, grow, proliferate and differentiate into target tissues. The latest development of water soluble PANi, which can translocate inside the bacterial cells without disrupting their membrane (being able to destroy the cell) is a major advancement, as this means that PANi can be safely applied without destroying cells other than the target. Numerous *in-vitro* studies have been carried out suggesting the biocompatibility of PANi towards various cell lines. However, when it comes to *in vivo* evaluation, most of the studies are still limited to small animal models. Therefore, more understanding of PANi *in vivo* response is needed in higher animal models like sheep and porcine. This is even more important as most recently the WHO has categorised aniline as a probable carcinogen in humans. Therefore, more research is needed to understand the biocompatibility of PANi more precisely, e.g. which is the exact mechanism of PANi degradation and how the biological processes involved eliminate it from the body. This must be investigated in long term studies. Furthermore, a better understanding of the immune response that PANi materials trigger will also be helpful in improving its biocompatibility. It is now well established that scaffolds or implanted materials interactions with macrophages can be controlled by inducing properties in scaffolds/materials such as roughness, topography and mechanical properties, in addition to biochemical effects, to direct macrophages to pro-healing pathways rather than pro-inflammatory pathways, therefore more research needs to be carried out to understand this tuning of PANi scaffold properties to improve its biocompatibility. Overall, as covered in this review, in the past few years numerous advancements have been made with PANi, however more research is needed to fully establish it as one of the leading conductive materials of choice for TE applications. It is the hope of the authors that the present review will contribute to open further research avenues to meet the mentioned unmet challenges in PANi science and technology.

Data availability statement

No new data were created or analysed in this study.

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