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The electrically conductive scaffold as the skeleton of stem cell niche in regenerative medicine

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ABSTRACT

Stem cells with multipotent and self-renewal abilities play a vital role in regenerative medicine and tissue engineering. They can assist tissue reconstruction through specific differentiation and secretion of various bioactive macromolecules. More and more studies confirm that the cell-fate commitment can be manipulated via constructing a specific stem cell niche. The construction of specific niches with conductive materials (conducting polymers, carbon nanotubes and graphene) can promote stem cell differentiation towards electro-active lineages and emphasize the promising role of stem cells in electro-active tissue regeneration (e.g., nerve and heart). In this review, we summarize the commonly applied conductive materials for scaffold construction and evaluate their contributions in the regeneration of electro-active tissues.

1. Introduction

Tissue engineering with the aim of developing biological substitutes that restore, maintain, or improve damaged tissue and organ functionality, has been intensively studied in the past few decades [1]. The strategy of tissue engineering is to replace a defective organ with an artificial organ composed of stem cells and a bioactive matrix that functions as an extracellular matrix (ECM) [2]. Tissue engineering is an interdisciplinary and multi-disciplinary field that many factors such as stem cells, biomaterials and biologically functional moieties affect the outcome. Among these factors, stem cell plays a dominant role due to its capacity of self-renewal and differentiation into specialized cell types. In practice, the commitment of stem cells could be affected by their resident niches. The niches are composed of supportive cells and ECM components arranged in a three-dimensional topography in the presence of growth factor gradients and they serve to initiate signal transduction events, either alone or in synergy with cytokines [3]. In tissue engineering, the niches have been engineered in the aspects of components, topography and growth factor incorporation to induce the specific differentiation of stem cells. For instance, laminin-5 is a component of ECM and it was found that the isolated laminin-5 matrix could activate an extracellular signal-related kinase (ERK)-dependent pathway to induce an osteogenic phenotype in human mesenchymal stem cells (hMSCs) [4]. Additionally, growth factors in the stem cell niches can mediate stem cell migration, proliferation and differentiation. Through incorporation of epidermal growth factor (EGF), insulin and other epidermal induction factors in core–shell structured nanofibers, sustained release of these induction factors could promote 60% epidermal differentiation of adipose derived stem cells (ASCs) on the core–shell nanofibers [5]. Besides the components and incorporated growth factors, the topography of scaffolds can also affect stem cell specification. The fibrous scaffold with smaller diameter was found to increase neural stem cell oligodendrocyte differentiation [6]. Meanwhile, it was found that the aligned morphology fiber could greatly improve neurite extension and gene expression for neural markers [7]. Recently, Hideki Taniguchi and colleagues constructed a well-designed stem cell niche and utilized the human induced pluripotent stem cells (iPSCs) to generate a three-dimensional vascularized and functional liver for the first time [8].

Similar as the chemical, topographical and their combined cues in guiding stem cell fate, electrical stimulation and conductive materials have also been applied in electro-active tissue engineering to assist tissue recovery and regeneration [9–11]. Regeneration processes induced by electrical stimulation were observed in rats with sciatic nerve crush injury [12–14]. Likewise, the optimized electrical stimulation (3 V/cm amplitude and 3 Hz frequency) applied to the engineered cardiac tissues was found to improve the contractile behavior of the engineered cardiac tissue with high level expression of cardiac troponin-I and connexin-43 [15]. Similarly, the conducting polymers (polypyrrole (PPy) and polyaniline (PANi)) incorporated scaffolds were found to assist stem cell neural and cardiac differentiation with longer neurite formations and higher relevant protein expressions [16–18]. Carbon nanotubes (CNTs) and graphene that possess high conductivity, excellent stability and mechanical properties were also intensively studied in tissue engineering, especially in electro-active tissues [19,20]. In nerve, cardiac and bone tissue engineering, CNTs with nano-scaled roughness and excellent conductivity were proven to be sufficient to

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modulate early stage of stem cell lineage commitment without the aid of induction factors [21–23]. Besides conducting polymers and carbon-based nanomaterials, metallic materials (e.g., stainless steel and titanium) have also been applied as functional substrates to guide stem cell specific differentiation [24,25]. Clearly, the combination of electrical and topographical cues of the conductive scaffolds can be an effective technique to promote electro-active tissue reconstruction.

As a promising research field, the biomimetic materials approaches that provide chemical, mechanical, and topographical cues to promote tissue reconstruction [26–28], as well as applying conductive scaffolds for tissue engineering, especially for nerve, cardiac and bone tissue engineering have been reviewed [29,30]. However, to the best of our knowledge, the effects of electrical stimulation and electrically conductive scaffold on stem cell specific differentiations in regenerative medicine have not been well discussed. Therefore, in this mini review, we summarize the applications of conductive materials in regenerative medicine and evaluate the combination of stem cell and conductive scaffold in promoting tissue recover and reconstruction. We first introduce the background of stem cells and stem cell niches, followed by the summarization of commonly used conducting materials and their fabrication approaches. Further, the applications of the conductive scaffolds in stem cell specific commitment are discussed in detail. Considering the active studies with promising outcomes in this research field, this review will inspire more research opportunities to explore the mechanism of conducting materials in promoting stem cell differentiation.

2. Stem cell and stem cell niches

Stem cell is one of the most important elements in regenerative medicine because of its capability in self-renewal, proliferation as well as differentiation into specific cell lineages under appropriate conditions. Based on their growth stage, stem cells can be classified as embryonic stem cells (ESCs) and adult stem cells. ESCs can be derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation *in vitro* [31]. Adult stem cells can be found in a number of tissues including umbilical cord blood, which possess the ability to maintain and repair the tissue [32]. The human ESCs maintain the potential to form derivatives of all three embryonic germ layers (endoderm, mesoderm, and ectoderm) [33]. However, using ESCs encounters some problems related to safety issues such as ESC rejection and the risk of tumorigenicity as well as ethical and religious issues [34–36]. MSCs are the commonly used adult stem cells in regenerative medicine and they can be isolated from the bone marrow [37]. Unlike ESCs, MSCs were found to exert an immunosuppressive effect *in vitro* and *in vivo* [38,39] by acting on all immune effectors [40]. MSCs with the multipotent properties can differentiate into different types of cells (such as muscle [41], heart [42] and cartilage [43]) developing from the mesoderm. Moreover, MSCs were also able to differentiate into neural or epithelia cells which are not from mesodermic origin [44,45]. Interestingly, even the ultimate differentiated adult cells can be reprogrammed into cells with pluripotent state due to their nuclear plasticity and these cells are called induced pluripotent stem cells (iPSCs) [46]. In a recent study, Japanese researchers cultured human iPSCs in a well-designed niche with many growth factors and supplementary cells and successfully constructed a vascularized and functional human liver from iPSCs. This study highlights the possibility of constructing a specific niche to guide stem cell differentiation and even tissue formation [8].

Stem cell niche, which was first described by Schofield in 1978 [47], is a specialized environment within tissues that provides signals to stem cells to regulate their viability, proliferation and differentiation. It is composed of the stem cell itself, stromal cells, soluble factors, extracellular matrix, neural inputs, vascular network and cell adhesion components [48]. The secreted factors act as massagers to realize the communication in the niche. This process is essential for the maintenance of proper stem cell function and for the determination of the fate of stem cell [48]. Besides the niche components, other mediators such as electrical stimulation can also regulate stem cell differentiation. Yamada et al. found that mild electrical stimulation strongly influences embryonic stem cells to assume a neuronal fate [49]. Therefore, electrical stimulation, conductive scaffold and their combination could play an important role in determining stem cell fate in electrically active tissues including nerve, cardiac and even bone tissues.

3. Conductive materials

Conducting polymers (CPs) are a class of π-conjugated polymers with loosely held electrons in their backbones [50]. Within the unsaturated backbone, the delocalized π-electrons can move freely to form an electrical pathway for mobile charge carriers [51,52]. With good stabilities, conductivities, and feasible synthesis [53], CPs have been used in biosensors to entrap biomolecules [54] and in tissue engineering to assist the recovery of the tissues (such as nerve, cardiac and bone tissues) which are responsive to electrical stimulation [55–58]. Polyaniline (PANi), polypyrrole (PPy), polythiophene (PT) and poly(3,4-ethylene dioxythiophene) (PEDOT) are the widely used CPs in conductive scaffold construction and their chemical structures are depicted in Fig. 1. Among these CPs, PPy is the most investigated one and it can be synthesized by electrochemical oxidation of pyrrole in an appropriate electrolyte solution (such as H_2SO_4) on a platinum coated electrode [59,60]. Langer and colleagues are the pioneers to study the interfacing cells and conducting polymer by culturing mammalian cells on the PPy thin films [61]. Later, Williams and Doherty applied the PPy as a biomaterial in nerve tissue engineering. Their results suggested that the conductive scaffold was cytocompatible and could be used as a nerve guidance channel and as a material for carrying stimulation currents at the same time [62]. Another frequently used CP, PANi, can be polymerized from its monomer aniline both chemically and electrochemically. Chen and colleagues electrospun aligned poly(ε-caprolactone)/PANi (PCL/PANi) nanofibers and found that the aligned PCL/PANi could guide myoblast orientation and promote myotube formation (approximately 80% increase in myotube numbers) compared with random PCL scaffolds [63]. Similarly, Abdul Rahman et al. electrospun poly(L-lactic acid)/poly(aniline-*co*-m-aminobenzoic acid) (PLLA/P(ANi-*co*-m-ABA)) nanofibers and cultured human adipose derived stem cells (hASCs) on the scaffold. The carboxylic acid functionalized PANi copolymers have higher solubility than PANi and the acid group of the copolymers can also act as a “self-dopant” to avoid an external dopant and increase the biocompatibility of copolymers. They found that the composite nanofibres supported the cell adhesion and proliferation. After 1 week of cell culture, hASCs were well spread on the substrates with abundant focal adhesions [64].

Graphene and carbon tubes (CNTs) are also widely applied as conductive materials in bio-substrates to promote cell attachment and proliferation [65–67]. Graphene is a one-atom thick layer of the layered mineral graphite with many extraordinary physicochemical properties, such as super electric conductivity [68], excellent thermal conductivity [69], high surface area (theoretically 2630 m^2/g for single-layer graphene) [70], and strong mechanical strength [71]. Chemical [72–74] or thermal reduction [75] of graphite oxide (GO) and thermal decomposition of SiC wafer under ultra-high vacuum (UHV) are the two mass-production methods to produce graphene. GO has emerged as a precursor offering the potential of cost-effective, large-scale production of graphene-based materials [70]. Other methods such as exfoliation (repeated peeling) of highly oriented pyrolytic graphite were also applied to produce graphene sheet. However, the yield is too low and it is mainly applied in research labs [76]. Carbon nanotube is the rolled-up graphene sheet and it is first discovered by Iijima in 1991 [77]. CNTs are synthesized in a variety of ways, such as arc discharge [78], laser ablation [79], high-pressure carbon monoxide [80], and chemical vapor deposition [81]. Researchers have intensively explored the application of CNTs due to their excellent mechanical, electrical,