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Research review paper

Engineering cell alignment *in vitro*

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ABSTRACT

Cell alignment plays a critical role in various cell behaviors including cytoskeleton reorganization, membrane protein relocation, nucleus gene expression, and ECM remodeling. Cell alignment is also known to exert significant effects on tissue regeneration (*e.g.*, neuron) and modulate mechanical properties of tissues including skeleton, cardiac muscle and tendon. Therefore, it is essential to engineer cell alignment *in vitro* for biomechanics, cell biology, tissue engineering and regenerative medicine applications. With advances in nano- and micro-scale technologies, a variety of approaches have been developed to engineer cell alignment *in vitro*, including mechanical loading, topographical patterning, and surface chemical treatment. In this review, we first present alignments of various cell types and their functionality in different tissues *in vivo* including muscle and nerve tissues. Then, we provide an overview of recent approaches for engineering cell alignment *in vitro*. Finally, concluding remarks and perspectives are addressed for future improvement of engineering cell alignment.

Contents

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1. Introduction

Alignment has been widely observed at various scales in tissues and organs, from extracellular matrices (ECMs) (*e.g.*, collagen fiber bundles in ligaments and tendons (Diop-Frimpong et al., 2011), concentric ECM waves in bone (Lanfer et al., 2009), aligned cells (*e.g.*, vascular epithelium (Kissa and Herbomel, 2010), striated muscle cells (Gokhin and Fowler, 2013) and neuron cells (Pacary et al., 2012), to cytoskeletal fibers such as microfilaments in rod-like matured cardiomyocytes (Ieda et al., 2010). Cell alignment, which refers to spatial and oriented organization of cells (Zhu et al., 2005), plays a critical role in pattern formation during embryogenesis (Etemad-Moghadam et al., 1995), tissue maturation (Chew et al., 2008b) and regeneration growth (Hoehme et al., 2010). The formation of cell alignment *in vivo* is generally accompanied with differentiation (Aubin et al., 2010), proliferation (Mauriello et al., 2009) and the changes of physical cues in surrounding cell microenvironment (Roux et al., 2009). It can further lead to the formation of various alignments of subcellular structures, including cytoskeleton, plasma membrane, and cell-adhesion complexes (Hoffman et al., 2011a). In addition, cell alignment combined with proliferation, migration and secretion of structural substances determines the hierarchy of cells and tissues, providing the physical and mechanical properties, and special biological functions at tissue levels (Jeong et al., 2005). Aligned organization of cells also results in secretion and deposition of a highly anisotropic ECM, which is specific to tissue type and critical in determining tissue function (Friedl et al., 2012). Therefore, it is essential to engineer cell alignment *in vitro* to regenerate structured and functional tissue equivalents.

With advances in nano- and micro-scale technologies, various engineering strategies have been developed to engineer cell alignment, including mechanical loadings (*e.g.*, stretching, fluid shear stress, and compression), topographical patterning (*e.g.*, microgrooves, nanofibers), surface chemical treatment (*e.g.*, cell-adhesive/repulsive pattern), and a combination of these modalities. These engineering approaches promote cell alignment either through mechanical modulation on intracellular cytoskeleton or directive physical and/or chemical gradients within local ECMs. Cells respond to these external stimulations and undergo an adaptation process, during which synchronization may exist in cell communications and signaling diffusions. Such an adaptation often leads to cellular cytoskeleton reorganization, directional cell spreading and growth, providing a necessary modification towards engineering densely packed, uniformly aligned cellular hierarchy at tissue levels.

In this paper, we present a state-of-the-art review on the engineering of cell alignment *in vitro*, with a focus on muscle cells, vascular cells and neurons. First, we present several typical cell alignments observed *in vivo* (muscle and nerve tissues) and the functionalities due to these special cell morphologies. Then, we discuss current engineering approaches that have been utilized to achieve cell alignment *in vitro*, with a focus on the feasibilities of four aspects (*e.g.*, spatial and temporal course of cell alignment, robustness, biological applicability to various cell types, and potential to engineer cellular constructs). In addition, we list established methods for quantifying cell alignment (Xu et al., 2011). Finally, we highlight the challenges and future directions for engineering cell alignment *in vitro*.

2. Cell alignment in different tissues

2.1. Cell alignment in muscle tissues and vascular tissues

Cell alignment is known to play an important role in providing special structure anisotropy for maintaining muscle tissue function. For example, axially aligned cells are necessary for effective contraction of muscle tissues (Valentín and Humphrey, 2009), skeletal (Choi et al., 2008), cardiac (Sands et al., 2011) and tension resistant for tendons, ligaments, and blood vessels. Here, we mainly introduce cell alignment in vascular tissues and striated muscle tissues (Fig. 1A–B)

2.1.1. Vascular tissues

Blood vessels have a distinct structural organization that provides both flexibility (resilience) and tensile strength properties, which are necessary for the pulsatile flow of blood. Vascular SMCs are circumferentially arranged in the form of fibrous helix within vascular media, collagen fibers, stacked between bands of elastin, and discontinuous sheets of endothelial basement membrane (Fig. 1A). This alignment appears to fully exploit the intracellular contractile protein orientation such that maximal vessel contraction and dilation occurs over a comparatively small range of shorting and lengthening of vascular SMCs, respectively. Specifically, the oscillatory strain exerted by pulsatile blood flow ensures the formation of cell alignment during vascular remodeling and angiogenesis. *In vitro* studies have shown that strain-induced alignment of vascular SMCs was accompanied with alternations of two smooth muscle phenotypes, *i.e.*, contractile phenotype (the quiescent secretory phenotype, marked by abundant contractile proteins such as actin and myosin) and synthetic phenotype (the growing mobile phenotype, marked by ECM deposition and actin synthesis) (Chan-Park et al., 2009; Diop and Li, 2011). Basically, vascular SMCs show a contractile phenotype in a healthy mature artery and may switch to synthetic phenotype. This phenotype switching usually associates with a number of vascular disorders, such as hypertension (House et al., 2008), restenosis (Gosens et al., 2003) and vasospasm (Alford et al., 2011). Thus, it is of great importance to induce SMCs to a physiological morphology and phenotype when engineering a functional vascular graft *in vitro*.

Another example of cell alignment in vascular system is endothelial cells (ECs), which are highly oriented along the direction of vessel longitudinal axis. ECs respond to a complex dynamic environment including various chemical cues and biophysical stimuli induced by blood flow. It has been demonstrated that the exposure of ECs to laminar fluid flow shear stress (FSS) elicits the alignment of intracellular cytoskeletal components (*e.g.*, actin fibers and microtubules) and hence cell elongation and polarization parallel to the flow direction (Duan et al., 2008). Shear stress due to blood flow acting on the endothelium is critical to many vascular functions, including the vascular remodeling, the maintenance of anti-thrombogenic properties, the physiological control of vessel diameter, the alternation of vascular permeability, and the pathological consequence of cardiovascular disorders. (Pries et al., 2010; Yang et al., 2006). Both small GTPase Rho and integrins were found playing important roles in inducing EC alignment in response to shear stress cues. For instance, a variety of studies have shown that shear stress rapidly mimics conformational activation of integrin $\alpha v\beta 3$ in bovine aortic ECs, followed by an increase in its binding to ECMs (Tzima et al., 2001). The shear-induced new integrin binding to ECM induces a transient inactivation of Rho similar to that seen when suspended cells are plated on ECMs. This transient inhibition is necessary for cytoskeletal alignment of ECs in the direction of flow. Additionally, shear stress induced by blood may change when atherosclerotic occurs and the fluid drag force acting on vessel wall is mechanotransduced into a biochemical signal that results in changes in vascular behaviors (Hubbell et al., 2009; Munson et al., 2013; Ng and Swartz, 2003). For interventional therapies, the implantation of coronary stents is a relevant part of interventional procedures for