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# Nanofiber size-dependent sensitivity of fibroblast directionality to the methodology for scaffold alignment

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ABSTRACT

The sensitivity of fibroblast guidance on directional cues provided by aligned nanofibers is studied for scaffolds of successively smaller fiber sizes (740 ± 280, 245 ± 85, 140 ± 40, and 80 ± 10 nm) fabricated using mandrel and electrical alignment methodologies for electrospun nanofibers (~10° angular deviation (AD)), as well as nanoimprint methodologies for perfectly aligned fibers (0° AD). On aligned scaffolds of large fibers (~740 nm) cell directionality closely follows the underlying fibers, irrespective of the alignment method. However, on mandrel aligned scaffolds of successively smaller fibers the cell directionality exhibits greater deviations from the underlying fiber alignment due to the higher likelihood of interaction of cell lamellipodia with multiple, rather than single, nanofibers. Using electrically aligned scaffolds, fibroblast directionality deviations can be maintained in the range of nanofiber alignment deviation for fiber sizes down to ~100 nm. This improvement in cell guidance is attributed to molecular scale directional adhesion cues for cell receptors, which occur within electrically aligned scaffolds due to fiber polarization parallel to the geometric alignment axis of the nanofiber under the modified electric field during electrospinning. While fibroblast directionality is similar on electrically aligned vs. nanoimprinted scaffolds for fiber sizes >100 nm, cell directionality is influenced more strongly by the perfect alignment cues of the latter on ~100 nm fiber scaffolds. The scaffold alignment methodology is hence highly significant, especially for tissue engineering applications requiring sub-100 nm aligned fibers.



## 1. Introduction

Aligned nanofiber scaffolds are commonly applied in tissue engineering to provide directional cues for cell migration and to enhance regeneration of oriented tissues, such as peripheral nerve and ligament tissues [1–5]. Scaffolds composed of nanofibers of successively smaller size promote various functionalities of the cells, such as the expression of ligament-specific biomarkers for mesenchymal progenitor cells [6], enabling better cell differentiation and proliferation of stem cells [7] and improving the adhesion and growth kinetics of fibroblasts [8,9]. Although there is a general trend towards studying cell and tissue interactions on progressively smaller nanofibers, the impact of the degree of alignment of size scaled fibers has been more difficult to assess. Previous studies of fibroblast cell guidance on polymeric nanofiber scaffolds composed of poly(D,L-lactic-co-glycolic acid) (PLGA) [10] and poly (methyl methacrylate) (PMMA) [11] suggest that for equivalently aligned nanofibers of sizes <1 μm, nanofibers below a critical size (~700–1000 nm) were unable to provide effective guidance cues to cells. This may be attributed to the relatively large size of focal adhesion complexes [12,13] in comparison with the fiber size, resulting in weaker complexes due to the availability of fewer anchor points for cell adhesion on scaffolds of smaller nanofibers. However, recent work has demonstrated that even on nanopatterned surfaces containing fewer anchor points, the presence of finely spaced anchor points (<90 nm) can promote cell adhesion through effective recruitment of integrin proteins to enable the formation of more stable focal adhesion complexes [14,15]. Herein we aim to understand the role of the method of fabrication of aligned nanofiber scaffolds on their guidance characteristics. Specifically, do the highly directional cues provided by perfectly aligned nanoimprinted fibers (0° angular deviation (AD)) and molecular scale fiber polarization cues of electrically aligned electrospun fibers (~10° AD) enhance cell guidance, especially on smaller sized nanofibers approaching ~100 nm, presumably through promoting conditions for the formation of more stable focal adhesion sites.

Nanofiber alignment is usually enabled by rotating mandrel-based mechanical approaches [16] or by electrical approaches for

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