Shape-memory Activated Change in Scaffold Fiber Alignment Directs Stem Cell Morphology

Ling-Fang Tseng, Patrick T. Mather, and James H. Henderson
Syracuse Biomaterials Institute and Department of Biomedical and Chemical Engineering
Syracuse University, Syracuse, NY13244

Statement of Purpose: Traditional tissue engineering scaffolds are static physical structures that cannot simulate the dynamic complexity of the *in vivo* microenvironment. Shape memory polymers (SMPs) are a class of active materials that can be manipulated and "fixed" into a temporary shape by network chain immobilization. Subsequent heating can be used to trigger shape change and recovery of the permanent shape. We recently developed cell culture-compatible SMP substrates that directs cell behavior through shape-memory-activated changes in surface topography [1, 2]. SMPs have also been studied for their potential as shape-changing tissue engineering scaffolds [3, 4], but a scaffold that can be triggered to change shape under cytocompatible conditions with viable and attached cells has not previously been reported. Our goal was to develop a 3D SMP scaffold that can change shape and internal scaffold fiber alignment under cytocompatible conditions and to study the effect on cell orientation.

Methods: Electrospun scaffolds were fabricated from a custom-synthesized shape-memory thermoplastic polyurethane and employed as shape-changing scaffolds as follows. An electrospun scaffold with randomly oriented fiber architecture was uniaxially stretched in a dynamic mechanical analyzer to 100% strain at 60 °C (above *Tg*, glass transition temperature) and fixed at 0 °C in the temporarily elongated shape, which exhibited a strain-aligned fiber architecture. Human adipose-derived stem cells (hASCs) were seeded on the strain-aligned (active) scaffolds as well as on unaligned and aligned control (static) scaffolds, and cultured at 30 °C for 24 h. The temperature was then increased to 37 °C, triggering a change from aligned to unaligned fibers in the active group, while the controls remained unchanged. Cells were then cultured at 37 °C for an additional 24 h.

Cell body alignment was assayed by fluorescence imaging before and after thermal triggering. Cells were labeled with Phalloidin to visualize filamentous actin. Two-dimensional fast Fourier transform (2D FFT) image analysis was used to characterize cell body alignment. The degree of cell body alignment was determined by the amplitude of peaks in each FFT plot, with higher peaks corresponding to higher alignment in a principle direction.

Results: Viable cells remained attached on both active and static scaffolds before and after thermal triggering (data not shown). Shape-memory activated change in fiber alignment of the active scaffold altered cell body alignment, with no comparable change observed in the static controls. Before transition, cells cultured on the aligned fibers of the active scaffold demonstrated two distinct peaks in the FFT plot at 90° and 270° (Fig. 1a, left). After transition, cells lost their preferential alignment, with no prominent peaks in the plot (Fig. 1a, right) and corresponding reorganization of cytoskeletal filaments was observed (Fig. 1a, inset). In contrast, on the static unaligned and aligned controls, cells remained randomly oriented or aligned, respectively, both before and after temperature increase (Fig. 1b and 1c).

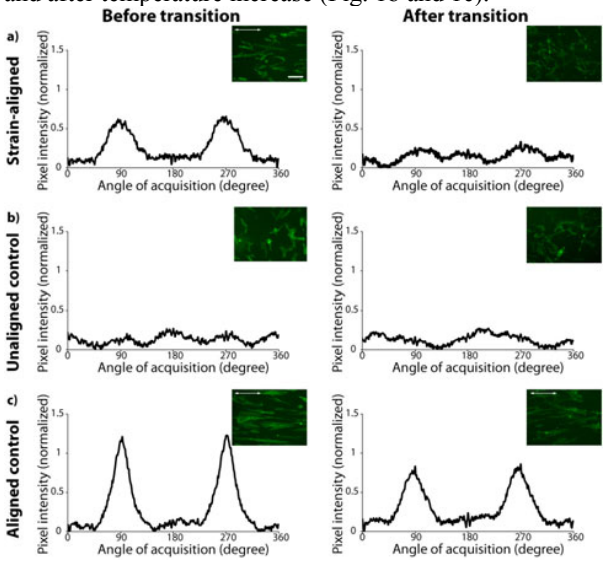

Figure 1. FFT analysis of the effect of scaffold fiber alignment on cell alignment. hASCs cultured on a) strain-aligned (active), b) unaligned (static), and c) aligned (static) scaffolds. Insets are representative fluorescent images of cell morphology. Scale bar = 100 μm. Double arrows in the insets indicate fiber alignment direction.

Conclusions: These results demonstrate a 3D SMP scaffold that can be triggered to change shape and internal scaffold fiber alignment under cytocompatible conditions with viable and attached cells. Quantitative FFT image analyses demonstrate that this active shape-changing scaffold is capable of controlling cell body alignment. SMP scaffolds are anticipated to provide powerful tools in cell biology and tissue engineering. For example, SMP scaffolds could be used to study the mechanobiological response of cells to dynamic changes in fiber alignment as occurs during tissue development, disease, and healing. In addition, SMP scaffolds could be used to address current limitations of tissue engineering scaffolds by employing dynamic programmable shape change to enable desirable functionality, such as shape-change-assisted cell seeding or minimally invasive delivery.

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