Thick collagen-based 3D matrices including growth factors to induce neurite outgrowth

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Abstract
Designing synthetic microenvironments for cellular investigations is a very active area of research at the crossroads of cell biology and materials science. The present work describes the design and functionalization of a three-dimensional (3D) culture support dedicated to the study of neurite outgrowth from neural cells. It is based on a dense self-assembled collagen matrix stabilized by 100-nm-wide interconnected native fibrils without chemical crosslinking. The matrices were made suitable for cell manipulation and direct observation in confocal microscopy by anchoring them to traditional glass supports with a calibrated thickness of ~50 μm. The matrix composition can be readily adapted to specific neural cell types, notably by incorporating appropriate neurotrophic growth factors. Both PC-12 and SH-SYSY lines respond to growth factors (nerve growth factor and brain-derived neurotrophic factor, respectively) impregnated and slowly released from the support. Significant neurite outgrowth is reported for a large proportion of cells, up to 66% for PC12 and 49% for SH-SYSY. It is also shown that both growth factors can be chemically conjugated (EDC/NHS) throughout the matrix and yield similar proportions of cells with longer neurites (61% and 52%, respectively). Finally, neurite outgrowth was observed over several tens of microns within the 3D matrix, with both diffusing and immobilized growth factors.

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1. Introduction
Cellular microenvironments, including the structure and rigidity of the substrate, topological and chemical cues such as growth factor concentration, orient the fate and behavior in physiological conditions. Smart devices for in vitro investigations of cellular events involved in their functions and in pathological situations must include a set of these stimuli associated with spatio-temporal control of their application to the cells [1,2]. The response to such cues gives an opportunity to orient the behavior of cells, favor a particular phenotypic engagement, and study specific aspects of their functions and dysfunctions. More specifically, a very active area of research is dedicated to the study and control of neural cell differentiation and, in particular, the outgrowth and guidance of neurite and the effects of distal events on the whole cell faith. Typical examples are retrograde signaling triggered by ligand-receptor interactions on distant neurites [3,4], or αβ-induced neurite degeneration followed by cell death [5]. Systems developed for this purpose most often rely on microfabrication techniques to design patterns with adhesion molecules or growth factor gradients [6–12]. Such patterned substrates are useful for studying the formation and branching of neurites, but also axonal dystrophies notably involved in neurodegenerative pathologies such as Alzheimer’s disease (AD). Several compartmented systems have also been developed with the aim of studying the response of neurites separately from that of the rest of the cell [13–20]. However, in most cases, the cells mostly evolve in a two-dimensional (2D) environment and/or the set-up does not permit the visualization in three-dimensional (3D) cell protrusions such as neuritic extensions.

The authors have developed a 3D biomimetic compartmented system suitable for the study of cellular events occurring within the matrix, near the tip of neurites. Macromolecules of the extracellular matrix (ECM) such as collagen, fibronectin or laminin are choice substrates for enhancing cell adhesion, and in some cases are required for the proper differentiation of specific cell types [21,22]. Most often, these adhesion molecules are deposited as a very thin layer onto the surface of glass or plastic dishes. The main drawbacks of such very thin coatings are that (i) the mechanical properties sensed by the cells are those of the underlying material (glass, plastic), which can largely modify the cells behavior [23] and (ii) the study of cellular events is mostly limited to two dimensions. Conversely, cells seeded on top of thick hydrogels consisting of either polysaccharides or proteins such as collagen [24–27] are...