The influence of incorporation of laminin peptide sequences on integrin activation and focal adhesion formation

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Statement of Purpose: As part of the central nervous system, the brain has a limited capacity to regenerate [1]; however, there are two regions in the brain called the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus that can give birth to new neurons [2]. Neural stem cells (NSCs) residing in these two regions have attracted increasing attention because of their potential for treating neurodegenerative diseases. Understanding the neurogenic niche provides the opportunity to optimize the effective factors promoting NSC differentiation toward neurons. SVZ niche is laminin-rich [3] which can provide some biological signals to affect NSC's behavior. Laminin can mediate cell attachment through different types of integrins including α6β1 and α3β1. Therefore, we are investigating the interaction of NSCs with laminin peptides immobilized on the glass substrate.

Methods: A laminin peptide (CGGGGAGQWHRVSVRWG) was conjugated to the maleimide-activated glass as shown in **Fig. 1A,** and Matrigel was coated on control substrates. Human-induced pluripotent stem cell-derived neural stem cells (iPSC-hNSCs) (ALSTEM, INC. Richmond, CA) were seeded onto substrates by applying manufacturer protocol with the seeding density of 10000 cells/cm^2. The multipotency of hNSCs was determined using immunocytochemistry (ICC) staining by evaluating nestin expression. The expression of integrin β1 was determined using capillary-based electrophoresis. Fourier Transform Infrared (FTIR) spectroscopy and X-Ray Photoelectron Spectroscopy (XPS) were carried out to characterize the peptide-modified substrates. To determine what range of peptide concentration could be used, different peptide solutions (10, 100 and 1000 μM) were added to substrates to be evaluated using XPS. The hNSC viability was then examined by staining with calcein-AM and ethidium homodimer-1 after 120 h. All data were reported as mean±standard deviation. One-way ANOVA with a Tukey post hoc test was performed at α=0.05.

Results: hNSCs were attached to the peptide-modified substrates and maintained their multipotency (**Fig. 1C**) compared with cells attached to Matrigel-coated substrates **(Fig. 1B**). It has been shown that cell attachment is due to the interaction of the AGQWHRVSVRWG peptide sequence with integrin α6β1 [4]. FTIR analysis showed characteristic bands of peptide bonds located at 1700–1600 cm^{-1} for C=O stretching vibration of amide I and at 3500–3300 cm^{-1} for N-H stretching vibration, indicating that the laminin peptides were immobilized to the surface (**Fig. 1D**). The functionalization of peptide-modified substrates was verified using XPS survey spectra, allowing the identification of individual elements. The expected peaks from C1s and N1s as characteristic elements were observed, confirming the peptides were covalently linked to the substrate. High-resolution XPS N1s and C1s core level spectra further verified their presence (**Fig. 1E**). Investigating the range of peptide concentration, three different concentrations of peptide solution were added to maleimide-activated surfaces. Using XPS, the atomic percentages of nitrogen (N) showed a significant increase with increasing peptide concentration, confirming the immobilization of peptides with different concentrations (**Fig. 1F**). To examine the viability of hNSCs on substrates, live/dead assay was performed. As it is shown in **Fig. 1G-N**, 2.5 nmol of peptide was not enough to support cell adhesion while 25 nmol and 250 nmol of peptide provided effective cell attachment. Our future work will focus on immobilizing two more laminin peptides and examining the α6β1 and α3β1 integrin activation and focal adhesion formation interacting with them.

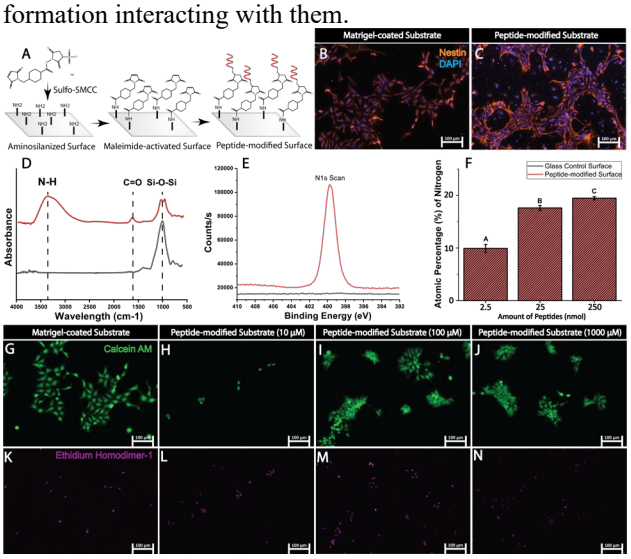

Figure 1. (A) The scheme of peptide surface functionalization. (B&C) ICC staining of nuclei (blue) and nestin (orange) after 3 days of culture to confirm hNSC's multipotency. (D) FTIR spectrum of glass control and peptide-modified substrates (N=3) to identify functional groups of laminin peptides. (E) high resolution XPS N1s of peptide-modified substrate (1000 μM) to indicate immobilization of peptides on the substrate. (F) Average N atomic percentage after addition of 10, 100 and 1000 μM peptide solution (N=3, n=3 & p-value: 0.000). (G-N) Live/dead assay after 120h using calcein-AM (green) and ethidium homodimer-1 (magenta) (N=3 & n=5) showing cell attachment and viability in groups with higher peptide concentration. Scale bar: 100 μm.

References:

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