Controllable Surface Expression of Bioactive Peptides Incorporated into a Silica Thin Film Matrix

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Mammalian cell culture platforms often require biomolecular modification to enhance cell adhesion and proliferation. Often, these modifications are performed using self-assembled monolayers or whole protein coatings, such as collagen. These protocols are inherently useful but generally suffer from repeatability. Undesirable conditions during self-assembly can lead to complications in the surface presentation of the biological ligands. Whole proteins are often unstable and derived from animal sources, making them less attractive for tissue engineering applications. As the biological effect of the material often depends strongly on the concentration of the integrated ligand(s), any complication due to synthesis or stability can lead to unexpected biological results. In this research, we expand upon previous work in peptide—silane modifications to sol—gel derived silica matrixes, demonstrating that the surface density of the peptide can be calibrated by simply modifying the starting liquid precursor concentration. The potential for calibration of peptide surface presentation allows for well-defined cell culture platforms that have the potential to mimic natural proteins in a stable, repeatable manner.

Introduction

Many biomaterial platforms designed to interface with mammalian cells in culture or tissues in biomedical implantable devices require bioactive peptides or extracellular matrix proteins to facilitate cell adhesion or direct cell differentiation.1–4 The biological effect of the material often depends strongly on the concentration of the integrated ligand(s). The ability to present multiple biomolecule ligands at defined and biologically relevant concentrations remains a major challenge. The hurdles are multifold and include a lack of data regarding native physiological concentrations and a lack of facile material preparation techniques. Previously, we demonstrated a peptide ormosil biomaterial platform that integrates rationally mixed peptides, covalently bound into sol—gel derived silica films.5 By altering the peptide mixture, designing it to interact with specific cell surface integrins, we were able to modulate the neuronal differentiation and neural cell phenotype distribution of retinoic acid differentiated P19 cells.5 The presence of the peptides on the surface was quantified using X-ray photoelectron spectroscopy through individual characterization using the carbon bonding structure of the molecule.7 Here we show that the surface density of the peptide can be calibrated by simply modifying the starting liquid precursor concentration. The result is a simple “one-pot” method for dialing in biomolecule ligand concentrations in solid-state thin films.

Current peptide-based materials are generally based on self-assembled chemistry, which requires several steps to complete.1,9–11 This several-step approach is reliant upon a critical atmospheric environment and can be easily hindered by undesirable conditions. These complications often lead to inconsistent surface chemistry between samples, potentially introducing unwanted differences between supposedly identical material samples. While for many experimental applications and studies this approach is convenient and sufficient, for biomaterials designed for implantable devices consistency and concentration regulation of material surfaces is critical. Biomolecule concentration differences less than 1 order of magnitude may lead to largely different experimental results. Additionally, the need to perform surface chemistry analysis on each implantable device or cell culture substrate is time-consuming, expensive, and inconvenient, if not impossible, on a manufacturing scale. Therefore, a consistent materials synthesis paradigm provided by the peptide ormosil platform offers benefit over traditional methods.

Numerous cellular processes operate under concentration or gradient-based interactions.11 Cell differentiation is dependent upon several factors, including spatial and temporal expression, as well as biochemical concentration. Molecular cues present in the developing cellular system are specific in their expression basis, and the concentrations are critical for the overall appropriate differentiation of the cell. The peptide ormosil system may offer increased benefit over traditional cell culture substrates, as it can be designed to interact with multiple specific cell receptors, depending upon the peptide ligands chosen, subsequently activating downstream cell signaling pathways. To direct differentiation of a cell population, the various signaling molecules must be present, at the appropriate concentration, and in a bioactive conformation. The peptide—ormosil platform has been shown to present cellular ligands at the surface through the peptide modifications; however, concentration-dependent expression at the surface has not been examined. The data presented here demonstrates the superiority of these
materials with respect to controllable surface expression of the peptides of interest.

Experimental Methods

Peptide—silanes were synthesized as described by Jedlicka. Two peptide silanes previously examined for surface presentation were chosen to determine concentration dependence of surface presentation. Hybrid peptide thin films were prepared with silica sol derived from tetramethoxysilane as previously described. Films were analyzed within 24 h postsynthesis. XPS spectra of the peptide—silica films were obtained by a Kratos Ultra DLD spectrometer using monochromatic Al Kα radiation (hν = 1468.58 eV). A fixed analyzer pass-energy of 160 eV was used for survey spectra, and the high resolution spectra were obtained at a pass-energy of 20 eV. The spectra were collected at a photoemission angle of 0° and 60°. A 60° photoemission angle was used to enhance surface sensitivity and verify surface real compounds. The surface coverage was calculated. The coverage was measured in monolayer (ML), which is defined as the ratio between the number of peptide molecules on the surface vs silicon atoms.

Results and Discussion

Two bioactive peptide—silanes were incorporated into a silica thin film at various concentrations to determine if the peptide surface coverage was concentration dependent. The first peptide—silane, ANDNIDPNAVAA, is simply denoted “NID”. The second peptide—silane is the ARDIAEIJKDIGA peptide, or “KDI”. The typical C 1s and N 1s core level spectra of the NID surface peptide prepared at a concentration of 0.5 mM/mL. The curve-fitting procedure has been verified. The difference between 0 and 60° photoemission angles mainly binding energy of a core-level depends on the chemical state of the atom. This phenomenon is referred to as a “chemical shift”. Based on the variety of the chemical states of the carbon atoms in a peptide, the constraint was designed assuming the Gaussian—Lorentzian shape of the individual component as shown in Figure 1. The curve fitting allowed us to quantify the amount of the surface peptide and to separate the contribution from the residual hydrocarbons. Peptide coverage dependence on concentration in a solution is shown in Figure 1. The plots in Figure 1 are based on the XPS data collected at a 0° photoemission angle. Coverage was calculated assuming that the surface peptide layer does not attenuate the XPS signal of the substrate (SiO2). This nonattenuating approximation was proposed by Fadley13 (see also discussion by Jedlicka7 for detail). The C 1s (after correction for the residual hydrocarbons) and N 1s14 signals were used for coverage calculations, and both approaches demonstrated remarkably consistent results. For both surface peptides, coverage dependence does not resemble a simple Langmuir type isotherm. A two-stage mechanism could be noted: the steep growth of the coverage is followed by gradual saturation (Figure 2). During the first stage, peptide coverage increases abruptly up to ca. 0.1 ML. The NID peptide exhibits a slightly lower growth than that of the KDI peptide. The final surface coverage achieved during the second stage is in 1.5 times higher for the KDI peptide than for the NID peptide. This indicates that the two peptides behave chemically different during the sol—gel processing. The KDI peptide is more hydrophobic, likely leading to a larger degree of intermolecular interaction during the processing. This might lead to higher final saturated coverage as well as the steeper coverage increasing during the first stage.

Analyzing the concentration dependence shown in Figure 2, we supposed that the turning point at ca. 0.1 ML (NID) and 0.15 ML (KDI) might correspond to the saturated coverage of peptides. Therefore, below saturation we treated the function of peptide coverage versus concentration as a simple Langmuir isotherm (see Supporting Information for calculation details). At low concentration, the slope of the curve is proportional to equilibrium constant, K1, which was 0.89 ± 0.18 and 1.14 ± 0.38 mL/mM for NID and KDI, respectively. The constant was obtained by averaging the coverage calculated based on the N 1s and C 1s peaks and for 0 and 60° photoemission angles. The difference between 0 and 60° photoemission angles mainly
results in a sizable experimental error. The scattering of 0 and 60° data are due to the sample roughness. Fadley’s formalism13 used to calculate the coverage assumes an atomically flat surface, but because our sol–gel derived silica films are not atomically flat we expect some difference between 0 and 60° data.

Above the saturation point, physical mechanisms begin to prevail. Multilayer adsorption and resulting bulk peptide interactions in the silica matrix lead to a large relative error in concentration calculations. The equilibrium constant, K2, estimated as the slope above 0.1 mM/mL was 0.13 ± 0.06 and 0.18 ± 0.10 for NID and KDI, respectively. This high relative error is likely a byproduct of increasing surface roughness from multilayer formation and the possibility of peptide agglomeration due to concentration-dependent biomolecular interactions. To investigate this possibility, an XPS analysis was performed after Ar+ sputtering of a peptide film. This analysis demonstrated the presence of carbon and nitrogen at a significant depth, whereas at low concentrations, the Ar+ sputter revealed very little carbon inside the silica films. Therefore, at low concentrations, the peptides are preferentially oriented at the surface of the material, and then at high concentration, the peptides create a highly doped multilayered structure.

Summarizing the results, we conclude that by varying peptide concentration, different peptide coverage can be achieved. This conclusion was made based on the XPS observation. There appears to be two competing mechanisms for the peptide integration into the silica thin films. First, at low concentration, the peptides orient preferentially at the surface and the thin films gel at a more rapid rate. In addition, given the high pore area to volume ratio, at low concentrations, the peptides that are located inside the pores of the structure are more likely to be spread homogenously throughout the material. The second mechanism is dispersion among the pores of the silica films. At higher peptide concentrations, it is assumed that more peptides will be found in any given pore, likely increasing the overall pore size and reducing the overall pore surface area.

Therefore, upon argon sputtering, there will be peptide still evident at the newly exposed surface. The higher concentrations of peptide–silanes will also likely form pockets of peptides at higher concentrations.7

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Supporting Information Available: The peptide coverage kinetics was treated as a simple Langmuir isotherm. This calculation is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


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