Characterization of Biomaterial Interfaces via Phage-derived Peptides

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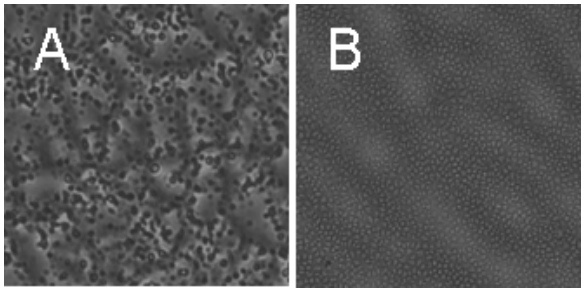
Statement of Purpose: The complex structural and chemical interactions occurring at the cell-material interface are extremely difficult to characterize. Advancements in understanding will require the development of new tools which convey chemical and structural information about the amount and bioavailability of functional groups on the surface.[1]

Background. Recently, combinatorial phage display has emerged as a powerful method to design peptides capable of selective and enhanced binding affinity to organic and inorganic materials including semiconductors, crystals, polymers, nanoparticles, and biominerals.[2] The phage display project is specifically directed toward the identification of highly specific peptidic species which bind to biomineral structures, polymeric materials and cell surface receptors. Successful characterization of the recognition and adhesive properties of the phage derived peptides presents opportunities to engineer these sequences onto quantum dots to develop new optical probes for imaging the chemical and structural species present both on material surfaces and cell membranes.

Results. In demonstrating the utility of this technique, we developed a biotin-based probe using a phage-derived peptide from the literature to identify the availability of RGD groups on the surfaces of genetically engineered silk blends provided to us through collaboration with David L. Kaplan of Tufts University. We synthesized the cyclic 7 mer peptide sequence, CWDDGWLC, published by Pasqualini et al in 1995 that served as an integrin receptor mimic for the cell-adhesive peptide, GRGDS.[3] We derivatized the N-terminus with a biotin group and following a surface incubation, used a gold nanoparticle derivatized streptavadin and a silver enhancement protocol to show that certain processing conditions caused the RGD sequences to be hidden from the surface.



RGD epitope presentation on the surface of the films was visualized through binding with an integrin mimicking peptide (CWDDGWLC-biotin) conjugated to streptavidin -colloidal gold (A). Annealing causes those epitopes to be buried such that they are not bioavailable (B).

In related work, using a phage display approach we have identified a peptide sequence which binds specifically to hydroxyapatite, (HA) Ca10(PO4)6(OH)2 , a polymorph of calcium phosphate HA is the primary inorganic component of both teeth and bone.4 Short peptide sequences capable of manipulating the crystallization processes while exhibiting specific and enhanced binding affinity to HA relative to other calcium phosphate polymorphs would prove invaluable in recognition, diagnostic, and therapeutic applications in vitro and in vivo. The peptide SVSVGMKPSPRP and corresponding phage were shown to bind to HA and the HA containing portions of a human tooth with low non-specific background. The identified peptide, showed selectivity to crystalline hydroxyapatite over amorphous calcium phosphate (aCP) and calcium carbonate (CaCO3). We are currently investigating using the specificity of the species to identify the progression of HA deposited during cell mineralization in situ and provide more information than the widely employed destructive endpoint measurements of the von Kossa assay or ortho-cresolphthalein complexone method which identify regions of high calcium Ca2+ content.

Surface vs. bulk phase separation is one of the great characterization challenges in polymer science. We have shown previously that composition dependent phase separation within a series of tyrosine-derived polycarbonates was shown to influence the acute inflammatory response and extracellular matrix production. These studies demonstrated that surface microstructure and topography strongly influenced cell attachment, spreading, and proliferation. One limitation was that because the topographical features of the surface were dependent on both composition and temperature, universal structure-function correlations regarding roughness, surface chemistry, and cell responses could not be readily ascertained. We are currently developing peptide based imaging probes for poly(DTE carbonate), one of the most promising library members, which will speed characterization of the phase behavior and enable structure property correlations. This presentation will highlight our latest efforts.

References

1. DG Castner, BD Ratner, Surface Science, 500, 28-60.
2. AB Sanghvi, KPH Miller, AM Belcher, CE Schmidt, Nature Materials, 2005, 4, 496.
3. R Pasqualini, E Koivunen, E Ruoslahti, J Cell Biology 1995, 130, 1189-96.
4. AS Posner, Crystal Chemistry of Bone Mineral, Physiol. Review 1969, 49, 760.
5. LO Bailey, ML Becker, JS Stephens, et al., J Biomed Mater Res A 2006, 76, (3), 491-502.