Bacterial Surface Display for Discovery and Study of Material Specific Peptides

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Statement of Purpose: Bacterial cell-surface display technology offers a powerful tool for the discovery and study of targeting bacteria to a specific material surface. Through peptide-material interactions, bacterial attachment can be tailored and binding fine-tuned for multi-component materials. Much of the work using bacterial cell-surface display technology has focused on biological interactions with protein systems, for therapeutic and biological threat sensing applications [1, 2]. Recently, however, we have shown this technology is more broadly extendable to other material sets, including a bulk aluminum alloy. A 15mer peptide named DBAD1 was discovered through biopanning with bulk aluminum [3] and a peptide library displayed on the surface of *E. coli*, known as eCPX [4]. Furthermore, we showed that the high aluminum binding affinity was likely facilitated by a peptide sequence-dependent, structure-function relationship with the target material. Computational analysis suggested that the helical backbone allowed the hydroxyl/sulphoxyl side-chain orientation on the same face towards the material in DBAD1[3]. This suggests that peptide-material binding maybe influenced by electrostatic interactions, leading to cross-binding with other similar metal oxides. It is therefore likely that DBAD1 may bind to other materials. The purpose of this work is to determine the specificity of DBAD1 to other common biomaterial sets to establish possible composition materials for cell adhesion patterning.

Methods: *E. coli* MC1061 cells harboring plasmid pB33-DBAD1 or pB33-NegLib were used. Cultures were grown in LB Miller broth supplemented with 25 µg/mL chloramphenicol at 37°C. eCPX display was induced with 0.04% arabinose and 2 mM EDTA (final volume). Cell adhesion to the material surfaces were measured using an indirect binding assay [3]. Briefly, after eCPX expression and surface display was induced, the various material samples were incubated with the cells. To remove unbound cells, the materials were washed in 1% PBS-Tween80 solution and the cells were propagated off the material surface by growth in LB with 0.2% glucose (final volume). Cell binding to each material was determined by sampling the number of cells in the LB-glucose by plating serial dilutions are LB agar. The materials used were 5.1 cm x 1 cm x 1 mm bulk aluminum alloy (Metals.com), 3.6 cm x 1.2 cm glass (Corning), 5.1 cm x 1 cm x 0.05 mm stainless steel (Shop-Aid, Inc), 5.1 cm x 1 cm x 0.13 mm copper (Shop-Aid, Inc), and 5.1 cm x 1 cm x 0.13 mm brass (K&S Engineering, Co). The indirect binding assay of *E. coli* cells expressing the DBAD1 peptide allowed adhesion to be compared across the five different materials sets and establish specificity of this peptide to the different materials. The inclusion of *E. coli* cells expressing eCPX scaffold alone (no peptide) served as a negative control for all experimental conditions. Duplicate, independent samples were employed and the average and standard error of the mean (SEM) calculated.

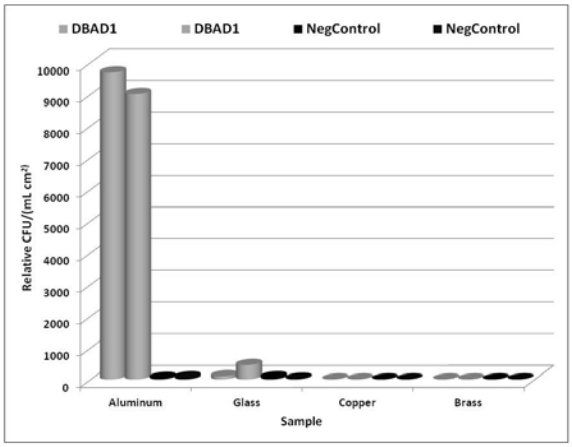

FIGURE 1. PEPTIDE FACILITATED BINDING OF *E. COLI* TO MATERIALS

Results: *E. coli* cells expressing the DBAD1 peptide on the cell surface bound best to bulk aluminum, as previously shown [3]. It was also demonstrated that DBAD1 did not bind strongly to glass, copper, or brass. There was an approximate order of magnitude difference in cell binding between aluminum and glass. Remarkably, there was little to no binding to copper and brass. These results, combined with essentially no binding exhibited by the negative control, strongly indicate it is likely that cell adhesion was due to the presence of the DBAD1 peptide.

Conclusions: The *E. coli* cell surface displayed peptide, DBAD1 was shown to preferentially bind aluminum, and stainless steel to a lesser extent. There was no binding to copper, brass, or glass. These results have important implications on the ability to direct microbial assembly on multi-component biomaterials. Future work will with DBAD1 and copper-specific peptides will used to demonstrate this capability.

References:

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