Characterization of Mammalian Cell Interactions on Coatings Based on Plant Polyphenols

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Statement of Purpose: Plant phenols and polyphenols [(poly)phenols] are ubiquitous secondary metabolites found in plants that are capable of performing a number of roles. These compounds are chemically versatile and connected to biological functions ranging from pigmentation to chemical defense, and from structural support to photo-protection.(Quideau S. Angew Chem Int Ed. 2011; 50: 586-621) Plant polyphenols have been celebrated by the general public and by scientists because of their many claimed health benefits, most notably antioxidant potential.(Stevenson DE. Cell Molec Life Sci. 2007; 64:2900-2916) Similar to the DOPA-rich adhesive proteins associated with marine mussels (Lee BP. Annu Rev Mater Res. 2011; 41:99-132) and sandcastle worms (Stewart RJ. J Polym Sci B. 2011; 49:757-771), plant (poly)phenols possess an abundance of dihydroxyphenyl groups (catechol). Interestingly, trihydroxyphenyl (galloyl and gallate) are also commonly found in these compounds. Accordingly, plant (poly)phenols also have strong solid-liquid interfacial properties, an observation that motivated their use in leather tanning and the name of the corresponding class of molecules capable of performing this task (tannins). Inspired by the abilities of plant (poly)phenols, surface-independent coatings were recently reported.(Sileika TS. Angew Chem Int Ed. 2013; 52:10766-10770) These inexpensive coatings based on tannic acid and pyrogallol provide biocompatible surfaces that are ideal for mammalian cell culture. The resulting materials retain many of the properties associated with their soluble precursors.

Methods: Non-treated polystyrene multi-well culture plates were modified with fibronectin, poly(L-ornithine), Cell-Tak™, and (poly)phenols. For (poly)phenol coatings, wells were either filled with 1 mg/mL tannic acid (TA) or pyrogallol (PG) in 100 mM bicine with 600 mM NaCl at pH 7.8. After agitating the solutions for 48 h, the plates were rinsed thoroughly with deionized water. Tissue culture polystyrene was also used as a control due to its prevalence in cell culture. A variety of cells, including NIH 3T3 fibroblasts, HeLa cells, Lund human mesencephalic (LUHMES) cells, and RAW264.7 macrophages were seeded on the surfaces. Experiments were conducted in order to study attachment, viability, cell spreading, proliferation, and migration. Additionally, in separate tests, cells were studied for markers of reactive oxygen species.(Sileika TS. Angew Chem Int Ed. 2013; 52:10766-10770)

Results: Nanoscale coatings inspired by plant (poly)phenols were recently shown to form on virtually any substrate. By dissolving TA and PG in buffered saline at pH 7.8, autoxidation affords a process similar to quinone tanning and melanogenesis, which forms species of greater molecular weight that adsorb on sample surfaces. The ability to form these coatings on most substrates was attractive due to the potential for creating a biocompatible interface on the wide range of materials used in biomedical applications. In order to characterize the interactions of mammalian cells on (poly)phenol coatings, multi-well culture plates were initially coated. As tissue culture polystyrene (TCP) is one of the few surfaces that cannot be modified by (poly)phenol coatings, culture plates composed of unmodified polystyrene were coated with TA and PG. In addition to showing excellent viability, NIH 3T3 fibroblasts were found to proliferate better on (poly)phenol-based coatings than on TCP (Figure 1). Additionally, the protective effects that are hallmarks of soluble (poly)phenols are maintained in these coatings. The antioxidant nature of (poly)phenols, which is linked to the ability to donate a hydrogen atom or an electron, is most associated with their reported health benefits. The coatings derived from TA are able to scavenge radicals and mitigate reactive oxygen species in chemical assays. Further, cells cultured on (poly)phenols films were able to overcome being challenged by soluble, pro-oxidant molecules. Similar behaviors were observed with HeLa cells, LUHMES cells, and RAW264.7 macrophages.

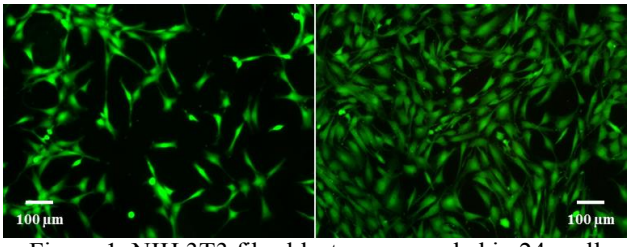

Figure 1. NIH 3T3 fibroblasts were seeded in 24-well plates at 7500 cells/well. After 24 h, cells were stained with calcein-AM for live cells and ethidium homodimer-1 for dead cells. (Left) Bare tissue culture polystyrene. (Right) TA-coated polystyrene.

Conclusions: We have demonstrated that coatings inspired by plant (poly)phenols can form on virtually any material. These surface modifications offer an interface that is both accommodating and protective for mammalian cells. In addition to promoting cell attachment, spreading, and proliferation, coatings based on tannic acid and pyrogallol were able to minimize the cellular production of reactive oxygen species. Given the coating versatility and biological properties of (poly)phenol coatings, we believe that the flexibility offered by these materials is immense, with potential applications involving cell culture studies and biomedical implant treatment. Future work will focus on discovering other coating molecules and investigating the anti-inflammatory potential of these bio-inspired films.