Interfacial Polymerization for the Amplified Labeling of Poorly Expressed Proteins

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Statement of Purpose: Interfacial polymerization in response to protein expression is a novel approach to the amplified fluorescent labeling of poorly expressed antigens. Polymerization initiators are immobilized at antigenic sites through specific biological interactions, and a hybrid polymer network consisting of fluorescent nanoparticles and PEG diacrylate is locally formed through interfacial photopolymerization.

Methods: Fixed permeabilized endothelial and fibroblast cells on glass slides are labeled with corresponding primary antibodies and biotinylated secondary antibodies. Cells are then incubated with a photoinitiator (eosin) conjugated to streptavidin. An aqueous monomer solution containing PEG diacrylate, vinyl pyrrolidone, methyl diethyl ether, and fluorescent nanoparticles (Invitrgoen Fluospheres) is introduced to the prepared sample. The monomer coated sample is then exposed to 500-650 nm light 30 mW/cm^2 for 20 minutes. Samples are rinsed with water and imaged. High dilutions of primary antibodies against high yield targets are used as a mimic of proteins of low expression.

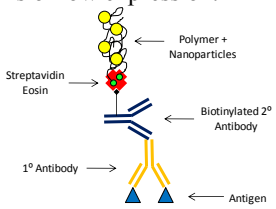

Figure 1. Schematic description of the polymerization labeling approach.

Results:

Dilutions of antibodies against vimentin were used on endothelial cells, and the expression labeling was amplified with polymerization based amplification. The resulting fluorescent hybrid film is readily imaged at high primary dilutions 1:50,000 (Figure 2). Additional results will be discussed showing polymerization amplification signal intensity comparable to that of enzymatic amplification, while lacking non-specific signal due to endogenous enzymes or signal diffusion.

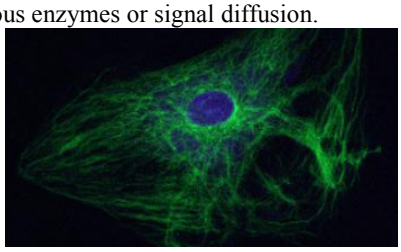

Figure 2. Polymerization labeling (green) of vimentin in human endothelial cells using 1:50,000 dilution of primary antibody.

The ability to stain against multiple targets is a requirement of any new labeling technique. We have demonstrated signal amplification against multiple targets in a single sample through sequential reactions. Human fibroblasts were fixed and permeabilized, and were incubated with antibodies against nuclear pore complex, biotinylated secondary antibodies, and the streptavidin-initiator conjugate. The nuclear pore complex signal was amplified through polymerization with Nile red nanoparticles and PEG diacrylate. The same sample was rinsed and incubated with antibodies against vimentin, biotinylated secondary antibodies, and the same streptavidin-initiator conjugate. The vimentin signal was amplified by polymerization with yellow/green nanoparticles and PEG diacrylate. The results shown in Figure 3, and appropriate controls indicate the continued availability of antigenic sites after polymerization amplification, as well as a lack of non-specific signal.

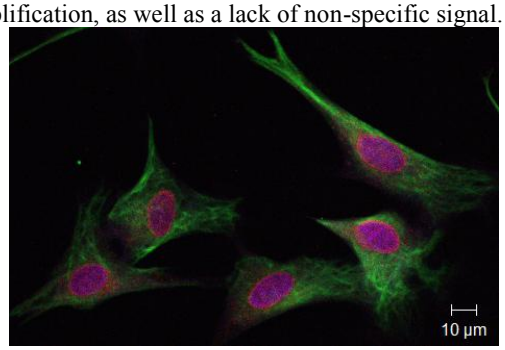

Figure 3. Polymerization based labeling of nuclear pore complex (red) and vimentin (green) in human fibroblasts.

Conclusions: *In situ* polymerization of an interfacial polymer film is a novel approach to the amplified labeling of protein expression. The amplification system is capable of labeling a low number of antigen/antibody binding events and is not limited by endogenous enzymes or signal diffusion. The specificity and conservation of antigenic sites makes this approach compatible with other labeling techniques. Additionally, this technology is a convenient approach to the formation of novel hybrid, nanoscale polymer films in unique geometries.

References:

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