A Photolinker Polymer with High Density Functional Groups for more Sensitive Bioassays

H. Gao, D. Caminada

CSEM's proprietary photolinker polymers are applied to develop covalently modified surfaces that can have additional (bio-)functionalities. Two developments enlarge the family of these photolinker polymers and extend their use for applications such as coatings for woundpads and catheters. First, the number of secondary functional groups to bind biomolecules has been increased and results in more sensitive bioassays. Next, a non-biodegradable organic photolinker (OptoBOD) has been developed to offer covalent coatings of increased stability and hydrophilicity.

The OptoDex technology is a versatile platform for covalent surface bioengineering and has been applied in diagnostics applications such as functionalization of biochips and microarrays. The degradable nature of the polysaccharide chains limited or even prevented its use for long-term stable coatings such as coatings for catheters and woundpads. We have developed a non-degradable organic polymer-based photolinker branded OptoBOD by replacing the dextran-backbone by a polyethylenimine-backbone. It maintains the advantages of OptoDex and increases the stability and hydrophilicity of the coating. OptoBOD addresses the needs for long-term and non-biodegradable coatings. The organic composition of OptoBOD makes it highly dissolvable in organic solvents, thereby allowing a more efficient coating process.

Biomolecules can be immobilized in two ways with our photolinker polymers: 1) by a dry-state photobonding process and 2) by secondary functional groups (e.g. $-NH_2$, -COOH, -SH, -Biotin, -Alkanes). Some biomolecules cannot stand a dry-state and only the latter approach can be applied. We have increased the numbers of available functional groups two- and four-fold compared to the first generation of OptoDex. The higher surface density of biomolecules on these two photolinkers results in a higher bioactivity.

The characterization of OptoBOD® included the determination of photoreactivity, binding efficiency, biocompatibility, surface property and stability. The UV spectra of OptoBOD® are recorded before and after irradiation to detect the photoreactivity. The decrease of specific adsorption at 365 nm indicates that the polymer labelled with photoreactive groups were activated by UV light, thereby generating carbene radicals for covalent crosslinking (Figure 1).

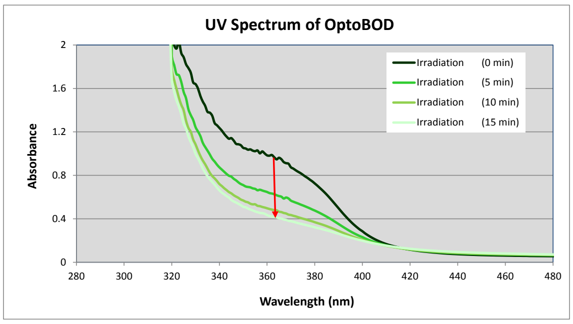

Figure 1: The UV spectra of OptoBOD® before and after irradiation, decreasing the adsorption at 365 nm, i.e. the specific wavelength of the photoreactive groups.

The binding efficiency of the photolinker polymers and the bioactivity of bound biomolecules were characterized by: i) Photoimmobilization of peroxidase on polystyrene (microtiter plate) and determination of enzymatic activity. ii) Photoimmobilization of monoclonal Anti-Peroxidase antibody onto polystyrene (microtiter plate), formation of immuno-complex by binding of peroxidase, and determination of enzymatic activity. The results of both tests (Figure 2) demonstrate an efficient light-dependent covalent binding of biomolecules and that the immobilized biomolecules maintain their bioactivity. However, the background signal increased with increasing numbers of functional amino groups available on the polymer. This is caused by the enriched positive charges on the surfaces (Figure 2).

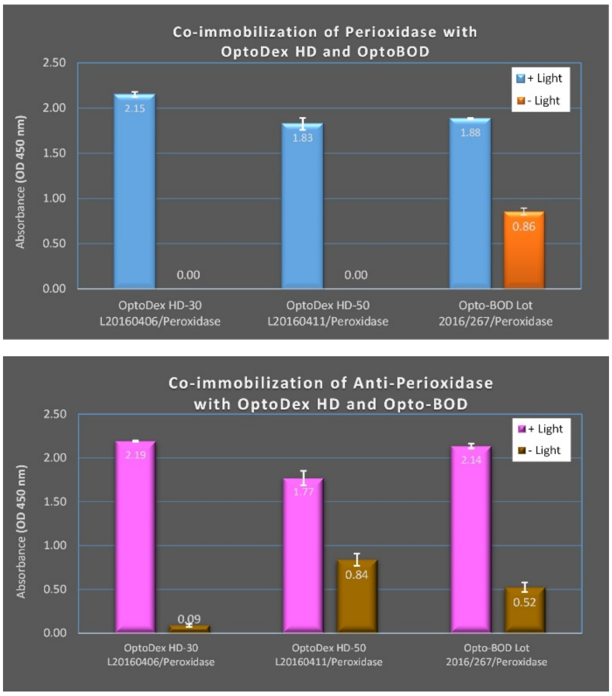

Figure 2: Photoimmobilization of peroxidase (top) or Anti-Perioxidase (bottom) on polystyrene (microtiter plate) and determination of remained enzymatic activity directly or after immuno-complex formation. Non-irradiated samples were used as controls.

The changes of material surface properties have been measured before and after coating with the photolinker polymers by contact angle measurement. All newly developed photolinker polymers (OptoBOD, OptoDex with high density functional groups) improve the wetting properties of materials (data not shown), i.e. the functionalized surfaces were more hydrophilic than the bare surfaces.

The two developments described here enlarge the CSEM family of photolinker polymers. They will allow to develop surfaces of more potent bioactivity, of higher stability, and of increased hydrophilicity.