Surface Modification Chemistries and Their Utilization in Microarrays, Diagnostics and Drug Delivery

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ABSTRACT

Completion of the human genome has resulted in a paradigm shift in the way biological queries are addressed today. Things that were considered far-fetched only a few years ago, such as personalized medicines, seem to be within our reach. New informatics and research tools have been devised to handle and analyze the influx of a huge amount of data, such as gene chips and microarrays or newly introduced nanoarrays. These devices are used to monitor biochemical and molecular interactions in a highly parallel and automated fashion. Such applications are also multiplexed on microspheres for diagnostic purposes. Because of the advantages of high throughput and high sensitivity, arrays and microspheres are fast becoming indispensable tools in diagnostics, drug discovery, clinical trials and other areas. Surface chemistry is one of the key factors to consider in successfully making sensitive and robust devices. Surface not only controls the anchoring of biomolecules, but also affects molecular interactions. SurModics uses its PhotoLink® photochemistry, to modify surface characteristics of devices for numerous applications, such as biomolecule and cell attachment, drug delivery, passivation of the surface, hemocompatibility and gene therapy. Surface coatings vary from sub micron to several microns in thickness. Such surface modification could also improve the performance of nano devices, such as biological micro-electromechanical systems, biosensors and microfluidic systems. SurModics has also used its surface modification technology to provide local, site-specific drug delivery from medical devices. The intent of combining drug delivery with the device is either to enhance the function and biocompatibility of the device or to simply serve as a vehicle for efficient drug delivery to a targeted tissue location. Unique formulations of a variety of polymer coatings have been combined with drugs to achieve stable, uniform films on medical device surfaces. After implantation, the surface film releases drug locally to provide the therapeutic benefit in the vicinity of the medical device.

Keywords: PhotoLink®, microspheres, medical devices, biomolecule attachment, multiplex assays.

1 INTRODUCTION

Surface modification is the process of changing the surface to possess more desirable and advantageous properties. Such properties are related to making the surface hydrophilic, hydrophobic or biologically more acceptable for diagnostics and medical devices. Surface coating is the interface between biology and device, thus providing the opportunity to tune it for the most suitable outcome. For example, mammalian cells grown on various substrates and bearing different properties, will result in morphological and gene expression changes in the cells. Similarly, a bare metal or plastic device implanted in the body will trigger immune and fibrotic responses. These responses will eventually result in fibrosis of the device rendering the device ineffective. Thus, protecting the device by passivating its surface or making it hemocompatible, is imperative. Gene expression and diagnostics analyses use small glass slides, microspheres or multi-well plates coated with suitable reagents to immobilize DNA or proteins and capture interacting biomolecules from blood, serum or purified samples. Achieving a high density of biomolecule attachment along with minimum non-specific binding is the key for a successful and reliable multiplex system. Contrarily, local drug delivery requires controlled release of pharmaceutical compounds from the coated reagents that are either bioabsorbable or biostable.

2 SURFACE MODIFICATION TECHNOLOGIES

SurModics uses its patented photochemical PhotoLink process to covalently couple surface-modifying reagents to surfaces. Such an approach could be used on preformed polymeric materials of any shape, and high-energy intermediates are capable of reacting with a variety of substrates. Surface-modifying photoreactive coating reagents can be divided into two groups: heterobifunctional reagents and multifunctional reagents (Fig. 1). Heterobifunctional reagents are mainly used to attach biomolecules to the surface, whereas multifunctional reagents are used to change surface properties, i.e., to achieve wettability or passivation. Anderson et al. [1] provide a detailed description of the technology.

The coating process is fairly simple and consists of two steps: application of the photoreagent by spraying or dipping followed by UV illumination under wet or dry conditions. No further steps are necessary.

Following are some of the advantages of the photochemical coupling:

1) Covalent coupling to any substrate containing hydrocarbon groups (metal and glass could be coated after application of a tie layer)

2) Variable coating thickness for multiple applications – monolayers to several micron
3) Environment-friendly coating materials. Several reagents are used in FDA approved devices
4) Compatible with device and product manufacturing processes.

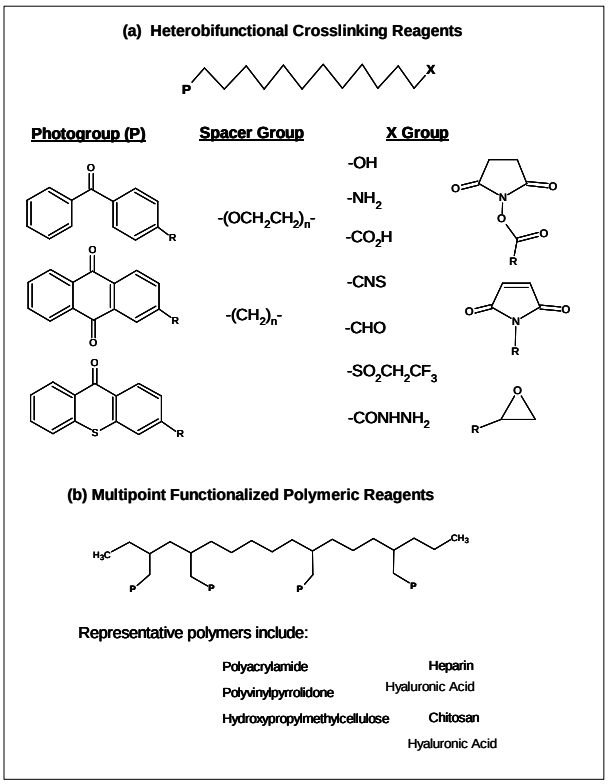

Figure 1: Photochemical reagents: a) heterobifunctional crosslinking reagents b) multipoint functional polymeric reagents.

3 APPLICATIONS OF PHOTOLINK COATINGS

Utilization of photochemical surface modification has been commercially successful in the following areas:

1) Hydrophilic surfaces – modification of the surface to make it wettable, lubricious or passivated. Important for devices for insertion and maneuverability and less adherence of proteins, cells and microorganisms.
2) Enhance blood compatibility of medical devices – these include heparin-based coating as well as non-heparin-based coatings with excellent antithrombogenic properties.
3) Antimicrobial coatings - to prevent device related infections by anti adherence properties or loading of antimicrobial compounds.
4) Local drug delivery – controlled release of pharmaceuticals from medical devices.
5) Diagnostics and biochemical assays – such as microarrays, microspheres or microfluidic devices.
6) Biomimetic surface modification – cell growth and tissue integration.
7) Tissue engineering – in situ formation of matrix directly at the site of interest such as cell encapsulation, wound healing and cartilage repair.

3.1 High Throughput Assays

Another important application of surface chemistry is for the modification of devices used for biochemical assays, such as slides for microarrays and microtiter plates for diagnostics, drug screening and cell-based assays. The substrates of these devices, mainly glass and plastic, are generally not suitable for attachment, interaction and analysis of biomolecules. The appropriate substrates are the ones that do not affect the structure and functions of the biomolecules attached or attracted to them, and also give a uniform and smooth surface for sensitive detection. Uncoated slides, microspheres or multi-well plates lack these properties that are essential for running multiplexed and highly sensitive assays. PhotoLink reagents are used to manufacture high quality microarray slides, CodeLink® slides (GE Healthcare, NJ). CodeLink slides are used for DNA, protein and antibody microarrays (Fig. 2).

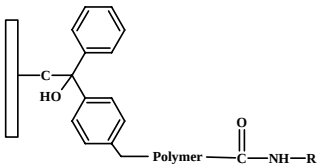

Figure 2: Photocoupling of polymers to glass surface for DNA and protein immobilization (R).

Following are some of the properties of the PhotoLink reagent, which make CodeLink slides a market standard.

3.1.1 Three Dimensional Configuration

Immobilization of biomolecules on a planar surface results in lower sensitivity and consistency. Membranous and polymer-coated substrates provide the porosity needed for higher loading of biomolecules as well as running the subsequent assays. Having a three dimensional supporting matrix is advantageous since it generates a uniform surface capable of homogeneous immobilization. Another advantage of having a 3-D configuration is that coating thickness can be varied for different applications. In a study in our laboratory with thicker-coated surfaces, 1.5- to 2-fold higher signal was achieved with the same concentration of oligos printed in a DNA microarray.

3.1.2 High Binding Capacity and Attachment Efficiency

Binding capacity is indicative of the reactivity of the surface conferred by active esters. Several factors affect this capacity, especially print buffers, thickness of the matrix and environmental factors. In an experiment in our laboratory, a

linear relationship was observed when a fluorescently tagged oligonucleotide was printed at various concentrations on CodeLink slides (Fig. 3).

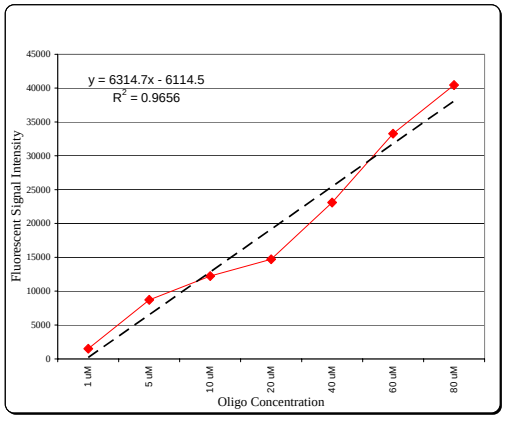

Figure 3: Signal intensity of an oligo printed at several concentrations. A 30-base long oligo was labeled with Cy3 on the 3' end and C6-amino linker on the 5' end. Signal was detected using an Axon scanner in appropriate channel.

3.1.3 Linear Dynamic Range and Sensitivity

Ramakrishnan et al. [2] assessed the performance of CodeLink slides for performing gene expression analysis in terms of specificity, reproducibility and dynamic range. Based on the transcript spiking experiment, sensitivity of the system was determined to be 1:900,000. Coefficient of variation of 10% was observed across slides and target preparation. Similarly, hybridization signal was linear over 2.5 to 3 orders of magnitude. These statistics indicate a very high quality matrix for running biochemical assays. Results have been corroborated in DNA-based hybridization assays as well as ELISA-based protein assays.

3.2 PhotoLink Coating of Polystyrene Microspheres

PhotoLink technology has been used to coat polystyrene microspheres with a very thin layer of polymer. The method has provided hydrophilic surfaces containing reactive groups without significantly affecting the size of the microspheres. The reactive groups have been used to further couple molecules for bioassays. These coatings were expected to provide improved biomolecule binding activity, low non-specific binding and better lot-to-lot reproducibility than currently available reactive microspheres.

In a study, polystyrene microspheres were coated with photoreactive, polyacrylamide polymers containing carboxylic acid functionality. Figure 4 shows SEM images of the photochemically modified microspheres compared to the unmodified microspheres (1.7 µm microspheres).

The functionality of the microspheres was evaluated by several methods. The first method was to evaluate the coupling efficiency of diaminopropane (DAP) to the carboxylic acid groups using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) coupling. Microspheres at a concentration of 14 mg/ml in 50 mM 2-(N-morpholino)-ethanesulfonic acid (MES) were reacted with EDC at a 1.3 mM concentration for 15 minutes. DAP was then added to give a final concentration of 3.7 mM and the reaction allowed to run overnight. After washing with PBS Tween and water, the microspheres were analyzed by ninhydrin assay. The amount of diaminopropane bound was 2.76 ± 0.08 µmole/g of microspheres (average of three measurements each from three lots of polystyrene microspheres). This result is compared to standard commercially prepared versions of acid-modified microspheres. It is estimated that the commercial microspheres contain 4 to 5 times more carboxylic acid (22 µmole/g) than the polymer-coated microspheres. The commercial microspheres were only able to bind 2.09 ± 0.02 µmole/g of diaminopropane in this experiment. The ability to adjust the reactive group load was demonstrated by coating with another polymer containing more acid functionality. The resulting microspheres in this case bound 3.83 ± 0.24 µmole/g.

Further evaluation was done by coupling of proteins to microspheres. Direct protein binding or ELISA results were similar to commercial carboxylic acid microspheres in flow cytometric analyses. However, binding of non-specific proteins to microspheres was half in the case of PhotoLink.

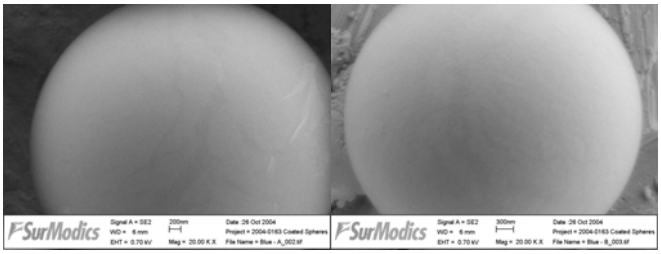

Figure 4: SEM images of microspheres with PhotoLink coating (Right) and uncoated (Left).

3.3 Matrix for Cell Culture

Mammalian cell culture is traditionally done in plastic containers that are coated with extra cellular matrix proteins, such as collagen or fibronectin. For applications such as therapeutic or vaccine production, these protein sources are not acceptable in cell culture. Numerous synthetic polymers and reagents have been suggested for growing cells *in vivo*, but huge variations in gene expression, adhesion, proliferation and migration have been discovered. PhotoLink polymers are used for cell and tissue culture applications. Since these are synthetic polymers, they do not have the same regulatory issues as animal proteins. Cells grown on such surfaces produce little stress fibers and exhibit good proliferation and morphology (Fig.5).

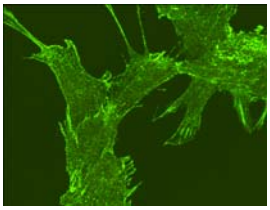

Figure 5: Human fibroblast cells grown on PhotoLink coated surface.

3.4 Drug Delivery Technology

SurModics is developing and commercializing several technologies for local delivery of pharmaceutical compounds. Early technology development has focused primarily on drug delivery coatings which are combined with medical devices to provide enhanced function of the device, such as a drug eluting stent. SurModics is also developing approaches to provide local delivery of drugs using devices or platforms whose sole function is to serve as a drug delivery vehicle. Because of the wide variety of available pharmaceutical compounds having diverse physical and chemical properties, SurModics is pursuing several strategies for controlling their release.

3.4.1 Biostable Coatings for Small Molecule Delivery

SurModics first developed coatings to provide controlled release of small molecules, primarily those that have low solubility in aqueous systems. We have used blends of biostable polymers to achieve coatings that provide tailorable drug release properties, while maintaining good mechanical strength and flexibility (see Figure 6 for an example showing control of drug release). One version of this type of coating is used on the commercially available

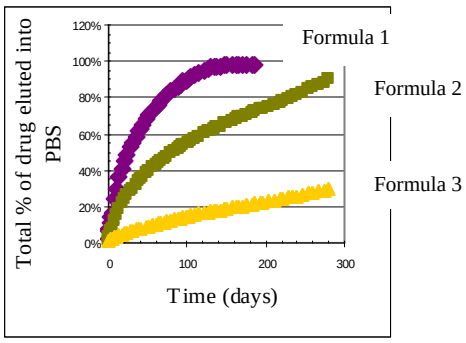

Figure 6: In vitro drug delivery from an ophthalmic implant

Cypher™ drug eluting stents. Recent advancements in coating technology at SurModics have been focused on coatings that can provide controlled release of highly water soluble small molecules, such as organic salts.

3.4.2 Biodegradable Coatings for Small Molecule Delivery

SurModics is also using biodegradable polymer technology to create coatings and matrices that provide controlled release of small molecule drugs. In the first phase of this development, SurModics is using PolyActive™ biodegradable co-polymers licensed from OctoPlus, a drug delivery company in the Netherlands. Coatings have been applied to the surfaces of stents and other substrates and have demonstrated favorable mechanical properties and tunable drug release rates. Development and fine tuning of these coatings continues.

3.4.3 Microparticle containing Matrices for Drug Delivery

Biodegradable microparticles entrapped in crosslinked matrices are another coating methodology used by SurModics to provide controlled release. This approach has several advantages, including allowing multiple drugs with different solubilities to be combined in the same matrix, and providing independent control over the release rates of each drug in the coating. For example, water-soluble and organic-soluble drugs contained in biodegradable microparticles and entrapped in a coating matrix have been shown to elute into aqueous media at rates independent of their solubility.

3.4.4 Biodegradable Coatings for Macromolecule Delivery

In further development of coatings at SurModics, biodegradable polymer technology is being used to create matrices which bind and release macromolecule drugs in a controlled manner. High molecular weight biomolecule drugs, such as peptides, proteins, oligonucleotides, and DNA can be controlled using these types of coatings and matrices.

4 CONCLUSION

PhotoLink surface modification technologies provide multiple surface properties and functionalities. These include simple passivation of surface to high-capacity biomolecule attachment and lubricious coatings to local drug elution. These modifications are imperative for diagnostics, gene expression and drug delivery through devices.

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