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# Fluorescent molecularly imprinted polymers as plastic antibodies for selective labeling and imaging of hyaluronan and sialic acid on fixed and living cells

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ABSTRACT

Altered glycosylation levels or distribution of sialic acids (SA) or hyaluronan in animal cells are indicators of pathological conditions like infection or malignancy. We applied fluorescently-labeled molecularly imprinted polymer (MIP) particles for bioimaging of fixed and living human keratinocytes, to localize hyaluronan and sialylation sites. MIPs were prepared with the templates D-glucuronic acid (GlcA), a substructure of hyaluronan, and N-acetylneuraminic acid (NANA), the most common member of SA. Both MIPs were found to be highly selective towards their target monosaccharides, as no cross-reactivity was observed with other sugars like N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-glucose and D-galactose, present on the cell surface. The dye rhodamine and two InP/ZnS quantum dots (QDs) emitting in the green and in the red regions were used as fluorescent probes. Rhodamine-MIPGlcA and rhodamine-MIPNANA were synthesized as monodispersed 400 nm sized particles and were found to bind selectively their targets located in the extracellular region, as imaged by epifluorescence and confocal microscopy. In contrast, when MIP-GlcA and MIP-NANA particles with a smaller size (125 nm) were used, the MIPs being synthesized as thin shells around green and red emitting QDs respectively, it was possible to stain the intracellular and pericellular regions as well. In addition, simultaneous dual-color imaging with the two different colored QDs-MIPs was demonstrated. Importantly, the MIPs were not cytotoxic and did not affect cell viability; neither was the cells morphology affected as demonstrated by live cell imaging. These synthetic receptors could offer a new and promising imaging tool to monitor disease progression.



## 1. Introduction

Altered glycosylation levels or distributions on the surface of cells are indicators of pathological conditions like infection or malignancy. Recent advances in glycobiology and cancer research have defined the key processes underlying aberrant glycosylations with sialic acids or hyaluronan in cancer and its consequences (Hascall et al., 2004; Fuster and Esko, 2005; Varki and Varki, 2007; Varki et al., 2009; Büll et al., 2014). Hyaluronan is a linear glycosaminoglycan composed of alternating units of D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc) (Fig. S-1, SI). Sialic acid (SA) is a generic term used to describe *N* and *O*-derivatives of neuraminic acid, of which the most common member is N-acetylneuraminic acid (NANA). Because the polysaccharides involved in the glycosylation procedure have a highly conserved simple composition and are ubiquitously expressed in all animals that have a developed immune response, they are so-called weak antigens. Therefore, production of antibodies that specifically recognize them is naturally difficult, and traditional immunohistochemical methods for detecting glycosylations on cells are rare (Kawamura et al., 1990; De la Motte and Drazba, 2011).

In this context, tailor-made molecularly imprinted polymers (MIPs) are promising synthetic receptor materials (Haupt et al., 2012; Piletsky and Whitcombe, 2013). Molecular imprinting is based on a templating process at the molecular level. Monomers carrying functional groups self-assemble around a template molecule (the target or a derivative), followed by copolymerization with cross-linking monomers, which results in the formation of a polymeric mold around the template. Subsequent removal of the template reveals three-dimensional binding sites in the polymer that are complementary to the template in size, shape and

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position of the functional groups. MIPs exhibit binding affinities and specificities comparable to those of antibodies. Their use as antibody mimics was first proposed by Mosbach's group (Vlatakis et al., 1993), and they are now sometimes referred to as 'plastic antibodies' (Haupt and Mosbach, 1998). In contrast to antibodies, their production is reproducible, relatively fast and economic, and no animals are necessary. Moreover, they are physically and chemically stable and are not degraded by proteases or denatured by solvents. Thus, MIPs have a great potential in providing a robust and specific imaging tool that reveals the location/distribution, time of appearance and structure of glycosylation sites on/in cells, which would lead to a better insight of the tremendously diverse biological processes in which these molecules are involved.

Very recently, we have published short communications on bioimaging of cells and tissues with MIPs labeled with organic dyes and quantum dots (QDs) (Kunath et al., 2015; Panagiotopoulou et al., 2016). To the same end, Sellergren's group has coated silica cores with a MIP shell containing nitrobenzoxadiazole as a fluorescent reporter group, to target SA on cell surface glycans (Shinde et al., 2015). Liu's group has reported SA-imprinted silica nanoparticles for surface enhanced Raman scattering imaging of cancer cells and tissues (Yin et al., 2015) as well as FITC-labeled silica particles with a shell imprinted with either SA, fucose or mannose to image these monosaccharides, overexpressed on cancer cells (Wang et al., 2016). Herein, we describe a more thorough study with MIPs labeled with organic dyes or quantum dots for multiplexed cell targeting and imaging, where we also show that single and dual-color imaging on live cells is feasible. To this goal, we synthesized fluorescently labeled molecularly imprinted polymers for imaging of human keratinocytes in order to localize and quantify hyaluronan and sialylation moieties on and in the cells. Since molecular imprinting of entire biomacromolecules like proteins or oligosaccharides is challenging, we opted for what is called the "epitope approach", which was inspired by nature (Bossi et al., 2007; Kryscio and Peppas, 2012; Bie et al., 2015). The monosaccharides, GlcA and NANA were used as templates to prepare the MIPs. Thus if GlcA and NANA are present and not sterically hindered, as for instance at the terminal end of hyaluronan or proteoglycans or glycoconjugates, they would be recognized and labeled. NANA is reported to be located extracellularly, at the end of sugar chains of sialylated proteins and sphingolipids on the glycocalix, whereas GlcA, apart from being extensively found in hyaluronan, is also present in some proteoglycans such as chondroitin sulfate, heparan sulfate and dermatan sulfate, though in lower proportions (Fig. 1) (Varki et al., 2009). Rhodamine-labeled MIP nanoparticles of size ~400 nm were synthesized in order to probe extracellular targets, and MIPs as a thin shell on InP/ZnS quantum dot particles with size ~125 nm were prepared for probing intracellular and pericellular hyaluronan (Evanko and Wight, 1999, 2001; Tammi et al., 2001).

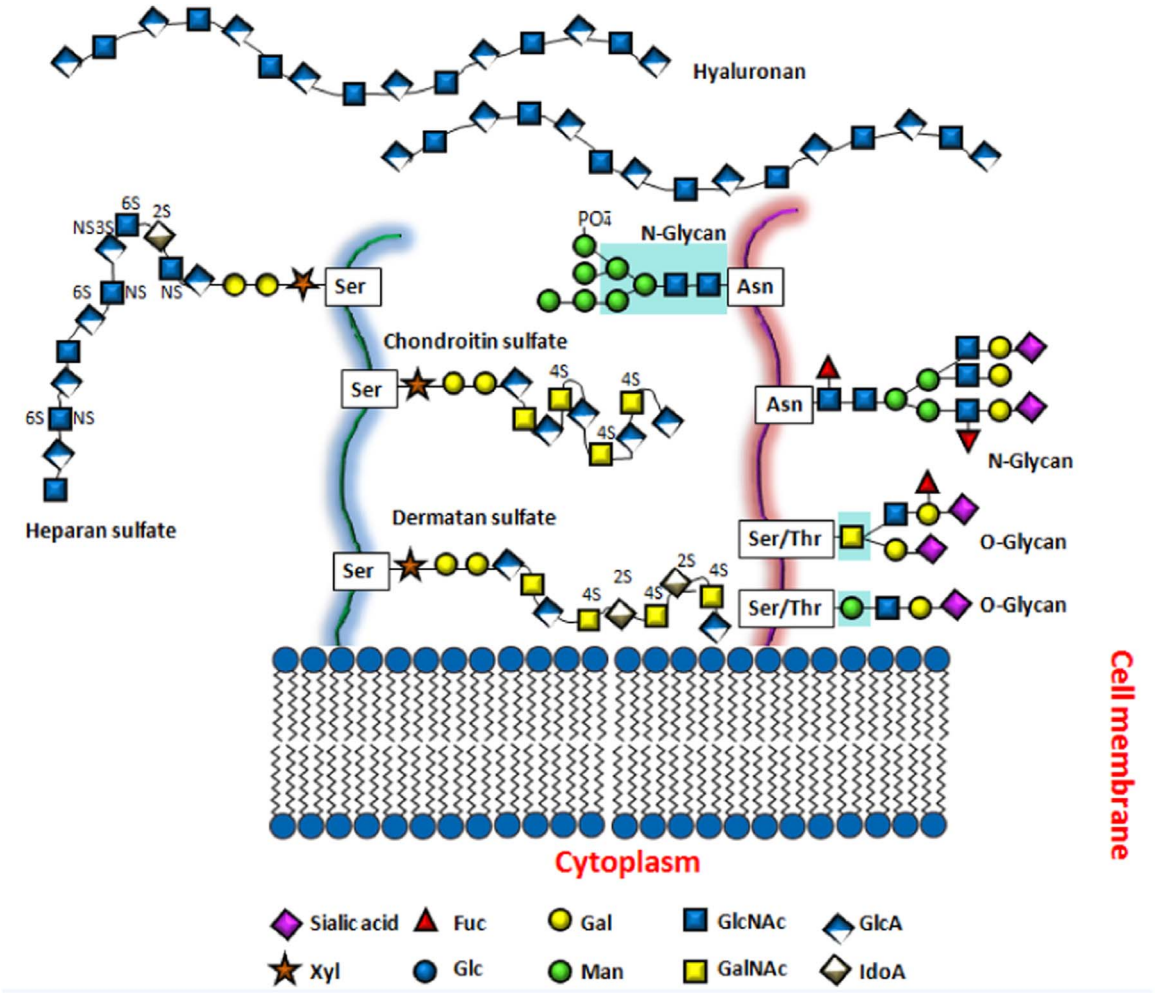


**Fig. 1.** The glycocalyx is a cell-coat structure of glycans and glycoconjugates that surrounds the cell membranes. Glucuronic acid (GlcA) is found extensively in hyaluronan and in smaller proportions, in dermatan sulfate, chondroitin sulfate and heparan sulfate while N-acetylneuraminic acid (sialic acid) is found at the terminal end of glycoproteins. Fuc: fucose; Gal: galactose; GlcNAc: N-acetylglucosamine; Xyl: xylose, Glc: glucose; Man: mannose; GalNAC: N-acetylgalactosamine; IdoA: iduronic acid. Adapted from Varki et al., 2009.

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