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Hyaluronate coating enhances the delivery and biocompatibility of gold nanoparticles

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ABSTRACT

For targeted delivery with nanoparticles (NPs) as drug carriers, it is imperative that the NPs are internalized into the targeted cell. Surface properties of NPs influence their interactions with cells. We examined the responses of retinal pigment epithelial cells, NIH 3T3 fibroblast cells, and Chinese hamster ovary cells to gold nanoparticles (Au NPs) in their nascent form as well as coated with end-thiolated hyaluronate (HS-HA). The grafting density of HS-HA on Au NPs was calculated based on total organic carbon measurements and thermal gravimetric analysis. We imaged the intracellular NPs by 3D confocal microscopy. We quantified viability and generation of reactive oxygen species (ROS) of the cells to Au NPs and monitored cell-surface attachment via electrical cell-substrate impedance sensing. The results confirmed that receptors on cell surfaces, for HA, are critical in internalizing HS-HA-Au NPs, and HA may mitigate ROS pathways known to lead to cell death. The 50- and 100-nm HS-HA-Au NPs were able to enter the cells; however, their nascent forms could not. This study shows that the delivery of larger Au NPs is enhanced with HS-HA coating and illustrates the potential of HA-coated NPs as a drug delivery agent for inflamed, proliferating, and cancer cells that express CD44 receptors.

1. Introduction

Gold nanoparticles (Au NPs) have been widely explored in medicine as potential delivery agents for various biopharmaceuticals (Kim, Kim, Jo, Yu, & Lee, 2011; Kumar, Raja, Sundar, Antoniraj, & Ruckmani, 2015). Although Au NPs are usually more biocompatible than other metal or metal oxide nanoparticles, their inherent positively charged surface disrupts the negatively charged cell membrane, while their production of reactive oxygen species (ROS) causes cytotoxicity (Moghadam, Hou, Corredor, Westerhoff, & Posner, 2012). Moreover, plasma proteins can spontaneously adsorb to nascent Au NPs, affecting the surface properties of the particles and their interaction with cells (Nel et al., 2009). As a result, surface modification is essential ideally to prevent protein adsorption (Larson, Joshi, & Sokolov, 2012), and non-specific delivery of Au NPs (Rana, Bajaj, Mout, & Rotello, 2012), to decrease opsonization by the immune system (Papasani, Wang, & Hill, 2012), and finally mitigate their toxicities.

Many coating conjugates, ranging from synthetic ligands to natural biomolecules, have been used to improve the stability of particles and their delivery to specific cells or tissues (Lin, Lee, & Shieh, 2017; Yilmaz, Demir, Timur, & Becer, 2016). Our focus is on hyaluronic acid (HA) as a coating material. HA is a bioactive linear polysaccharide that prevents adsorption of proteins on surfaces of biomaterials (Hans & Lowman, 2002) and has an antifouling effect that arises from its hydrophilic and polyanionic characteristics (Lee, Lee, Kim, & Park, 2008; Santhanam, Liang, Baid, & Ravi, 2015). HA also scavenges free radicals and chelates pro-oxidant metals (Glucksam-Galnoy, Zor, & Margalit, 2012). HA serves as a ligand for several cell-surface receptors and thereby has an important physiological role. Cluster of differentiation 44 (CD44) is the most well-studied cell membrane receptor (Jaggupilli & Elkord, 2012). It is present on, for example, the lymphatic vessel endothelial HA receptor (LYVE-1) (Chen, Cursiefen, Barabino, Zhang, & Dana, 2005), the receptor for hyaluronate-mediated motility (RHAMM) (Nedvetzki et al., 2004), and the HA receptor for endocytosis (HARE) (Pandey & Weigel, 2014). The type of HA receptor on the cell membrane depends on the function of the tissue.

The role of HA receptors in healthy and diseased cells is not well understood. CD44 had been identified during the development, differentiation, and proliferation of cells, for example, the neural retina and in Muller cells (Chaitin & Davis, 1995). CD44 has also been found under pathologic conditions, such as inflammation and proliferative vitreoretinopathy, where retinal pigment epithelial (RPE) cells actively

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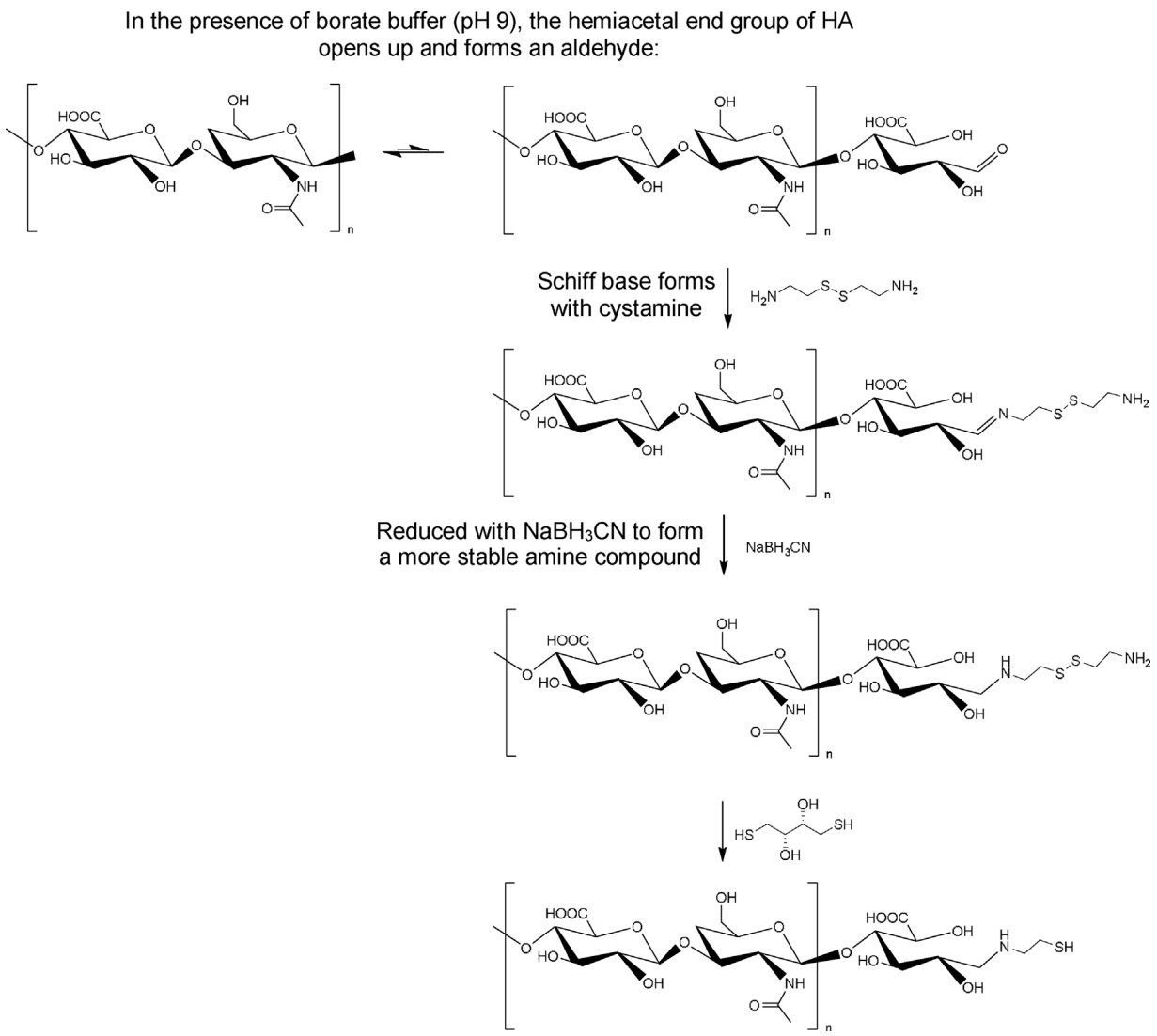

Fig. 1. HS-HA synthesis chemistry. In the presence of borate buffer at pH 9, the terminal cyclic hemiacetal ring opens and changes to a linear aldehyde form. A Schiff base forms with cystamine. Thus, the terminal saccharide reverses between two states to reach the final form. The Amadori compound, sodium cyanoborohydride ($NaBH_3CN$), is added, and then Dithiothreitol (DTT) is added to reduce the disulfide and produce HS-Has.

proliferate in the subretinal space, on the surface and undersurface of the retina, and in the vitreous cavity. Hence, RPE cells exhibit significantly higher CD44 receptors compared to their quiescent and stationary state (Moysidis, Thanos, & Vavvas, 2012). Furthermore, it has been reported that cancer cells also express CD44 receptors and have a significant regulatory role in almost all cancer types (Kesharwani, Banerjee, Padhye, Sarkar, & Iyer, 2015; Wang et al., 2016). It is now well recognized that a threshold of CD44 expression is required before HA binding is observed (Perschl, Lesley, English, Trowbridge, & Hyman, 1995; Tzircotis, Thorne, & Isacke, 2005). Therefore, cells like NIH 3T3, which express relatively less number of CD44 receptors, may lack HA binding while cells like Chinese hamster ovary (CHO) cells with high CD44 receptor density show strong HA binding (Katoh, Zheng, Oritani, Shimozato, & Kincade, 1995; Tzircotis et al., 2005).

In our previous study, we have found that for a fixed total surface area, Au NPs (particle diameter, dp < 50 nm) were toxic to retinal pigment epithelial cells, independent from the particle size (Karakocak et al., 2016). The toxicity of Au NPs arises from reactive oxygen species (ROS) activity, which was shown to be correlated to the available total surface area (Jiang et al., 2008; Pan et al., 2007). HA is a known free radical quencher; it is natural and consequently biocompatible (Balogh, Illes, Szekely, Forrai, & Gere, 2003). Our objective in the current work is to expand the usefulness of Au NPs by mitigating their toxicity via coating them with HA. As expected, we observed that HA coating significantly enhanced the biocompatibility of Au NPs; unexpectedly, the coating also greatly improved the internalization of larger Au NPs, which in their nascent form could not enter the cell. The outcomes of this study could be valuable in treating inflamed, proliferating, or cancer cells that express CD44 receptors.

2. Materials and methods

2.1. Reagents

1-Ethyl-3-[3-(dimethylamino)propylcarbodiimide (EDC), sodium citrate, hydrogen tetrachloroaurate ($HAuCl_4$), cystamine dihydrochloride, sodium cyanoborohydride ($NaBH_3CN$), Dulbecco's modified Eagles's medium/nutrient mixture F-12 Ham (DMEM/F12), trypsin-ethylenediaminetetraacetic acid (EDTA) solution 10x, and fetal calf serum (FCS), and DAPI for nucleic acid staining were obtained from Sigma-Aldrich (St. Louis, MO). ARPE-19 (Retinal pigment epithelial) (ATCC® CRL¬ 2302™), NIH 3T3 (ATCC® CRL¬ 1658™) and CHO (CCL¬ 61™) cells were purchased from American Type Culture Collection (Manassas, VA). Dithiothreitol (DTT) was obtained from Duchefa