Protamine/Heparin Detection with Nanosensors: From Research to Point of Care Device

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Heparin is a natural polyanion widely used as an anticoagulant during surgical procedures. As it is metabolized after injection in the bloodstream, minimizing the measurement time is critical to prevent clotting at too low concentration or uncontrolled bleeding at too high concentration. These needs are not compatible with the heparin quantification gold standard, the anti-Xa assay, because it requires sample preparation as fluorescence cannot be performed in whole blood, owing to its high background signal. Heparin effects may be reversed by addition of protamine, an arginine rich protein, via polyionic interaction.

Meyerhoff and coworkers achieved pioneering work on protamine and heparin detection since 1994 with ion-selective electrodes [1] and optical sensors [2]. More recently, our group developed protamine nanosensors with a solvatochromic dye as signal transducer [3]. The protamine ionophore dinonylnaphthalenesulfonic ($DNNS^-$) was found to strongly polarize the dye environment within the nanoparticles. With protamine binding, the dye interacts with a much more apolar environment, resulting in an absorbance shift. The particles response was found to be independent of pH, which is a significant advantage compared to typical optical nanosensors. Heparin was successfully quantified in patients' plasma and the mechanism of the nanosensors was investigated. However, this approach still required consequent sample preparation as it was not suitable for whole blood measurement. Thus, the nanosensors were embedded in an agarose gel, known to filter out the red blood cells, and poured in polystyrene cuvettes for absorbance measurement [4]. Protamine diffusion though the gel induced a color change, which was recorded with a camera. Heparin quantification in whole blood was achieved with this very simple setup. Yet, this was still far from a point of care device.

We report here on the development of a novel heparin assay that that combines paper-based devices and agarose gels to detect heparin in whole blood samples via protamine titration. The device is composed of multiple layer stacked onto each other. A few µl of sample is dropped on the top layer that traps the red blood cells to prevent color interference. The sample components then diffuse through a filter paper preloaded with protamine and enters a thin layer of agarose gel containing the nanosensors where unbound protamine induces a color change. A camera is placed below to quantify the sensor response and correlate it to heparin concentration. This avoids sample pretreament and provides a rapid assay for heparin. As the nanosensors function in exhaustive mode (taking up all protamine until saturation), adjusting their composition may tune the response to different heparin ranges depending on the desired application.

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