"Detecting Autologous Blood Transfusions Using Dielectrophoretic Spectroscopy"

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Project Overview:

he ability to increase oxygen carrying capacity to exercising skeletal muscles is a highly effective method for improving athletic performance. Unfortunately, some athletes seeking to gain an edge over their competition, have turned to artificially enhanced performance gains through blood transfusions, despite these methods being banned by the World Anti-Doping Agency. While methods such as flow cytometry can reveal heterogeneity in red blood cell (RBC) surface antigens and thereby detect homologous transfusions – or blood doping from a different person, there is currently no method available to detect autologous blood transfusions (ABT) with an athletes own blood. The lack of a direct detection method represents a significant problem for endurance sports, and the absence of a test means that this performance enhancing method is still widely utilized. There is evidence that biochemical changes occur in RBCs stored ex-vivo, including changes in their cell membrane that do not occur in a normal RBC population. One major obstacle to the development of a specific and reliable method for detecting ABT is then the lack of an available technique for detecting these age-related changes in circulation and at low concentration.

The goal of this proposal is to develop the ability to quantify these modifications in red blood cell storage age using a combination of dielectrophoretic spectroscopy and a storage sensitive membrane cross-linking reaction, and to employ this approach to develop a simple and specific test for the detection of autologous blood transfusions in endurance athletes. The successful outcome of this project will lead to the development of an entirely new electrical approach to monitoring an athlete's blood sample, and will lead to a new ABT indicator that is simple, rapid, require only a small droplet of blood, and capable of being integrated into an athlete's Biological Passport.

Results and Conclusions:

Background. The ability to increase oxygen carrying capacity to exercising skeletal muscles is an effective method for improving athletic performance. Unfortunately, some athletes have turned to artificially enhanced performance gains using blood transfusions, despite these methods being banned by the World Anti-Doping Agency (WADA). One main limitation in detecting autologous blood transfusions (ABT) is that there is no direct method capable of performing specific detection across a large transfusion regime, including detecting re-infusion with a small volume of blood under conditions when re-infusion has occurred multiple weeks prior to a

competition. The significance of this project is based on this effort to develop a new method to overcome this problem using a combination of electrokinetics and microfluidics.

Results. We used electrodes to measure the electrical behavior of RBCs. To perform our experiments, RBCs were collected from healthy human volunteers and stored in storage buffer. We discovered that the electrical properties of RBCs change when cells are stored. We also developed an ABT assay that can quantify differences in mechanical elasticity of RBCs based on the ability for RBCs to deform in a microfluidic channel.

Conclusions. We believe that these two ABT assays complement each other and speculate that the microfluidic deformability assay can be used as a rapid screen for athletes to detect potential doped subpopulations of RBCs. The electrokinetic assay could then be used as a secondary detection method to verify the presence of aged RBCs. We are looking forward to evaluating the performance of these assays on in future work from samples collected from doping volunteers.