**History and Features:**

BioMagnetic Solutions' magnetic products are a unique class of magnetic material – biologically active/compatible ferrofluids (FF). These ferrofluids were invented by one of our founders, Paul Liberti (Patent #s 4795698, 5108933). Based on their unique properties, he used them in his first biotech venture, Immunicon, for the isolation of circulating tumor cells which led to the development of CellSearch© [1]. The CellSearch© system, capable of detecting as few as five tumor cells in 1 mL of patient blood, was the first FDA approved system for monitoring breast cancer patients during therapy. Ferrofluids are indeed nanoparticles ranging in sizes from 40 to 140 nm, which makes them colloids, but they are very different from magnetic beads of the type formulated by John Ugelstadt and Robert Molday. Our nanoparticles are 84% magnetic mass whereas the others are typically 8%. The difference lies in how they are produced. In the case of the Ugelstadt's beads, polymerizations that result in their formation are done in the presence of iron salts which are subsequently converted to magnetite in a secondary process (Patent #s 4654267, 4774265). BioMagnetic Solutions' ferrofluids are made by first forming crystalline cores of magnetite which are subsequently coated with protein which is locked onto the cores by proprietary processes. For our 130 nm ferrofluids, crystalline cores of about 100 nm are first formed and subsequently coated with multilayers of bovine or human serum albumin to which monoclonal antibodies or other proteins such as streptavidin or other common capture agents are covalently coupled. The ferrofluids that we are using at BioMagnetic Solutions are a substantially improved version over those that were used for CellSearch. The coatings are more stable and they exhibit lower nonspecific binding.

There are two unique features of ferrofluids which are the consequence of their colloidal nature and their high magnetic moment. Because they are colloids, they react with diffusion control kinetics and require no mixing. That makes them ideal for targeting the complete spectrum of concentrations which in the case of cells ranges from rare cells (3-5 cells/$10^8$) to 70 – 80% of the population. Further, since colloidal solutions are uniform our ferrofluids can be pipetted with extreme accuracy. Because of their high magnetic moment, under the influence of a magnetic field, ferrofluids will form small chains of North-South nanoparticles. Such chains have synergistic magnetic properties making them readily separable in relatively low magnetic gradients, e.g. > 3-4 kGauss/cm. Consequently, ferrofluids or ferrofluid-labeled entities such as cells can be separated from vessels (microtiter wells, test tubes, etc) simply by placing them near an appropriate external magnet, with no need for high gradient columns. At very high concentrations ferrofluids exhibit properties of increased viscosity and density in a magnetic field forming magnetic phases (called ferrophases) – allowing for many other useful applications.

**Applications:**

These unique properties of ferrofluids, as well as the fact that these materials shift the NMR spectra of the hydrogens making them powerful contrast agents, lend themselves to many important applications in the biotechnology world. For example, they have been used in the construction of immune-based assays of the highest sensitivity. For high-sensitivity sandwich assays that can be done by incubating biotinylated capture monoclonal and a signal monoclonal with sample for 7 – 10 minutes followed by the addition of streptavidin ferrofluid. The secondary incubation only requires about four minutes after which the ferrofluid can be separated in a monolayer that can readily be freed of signal antibody without resuspension and where signal can be read directly from that

monolayer [2]. It will be recognized that this approach combines the best properties of solution reactions with those of coated cups where the formation of sandwiches is done in liquid phase and after separation the monolayered ferrofluid is processed as a coated cup (Patent # 5660990).

In the case of cell separations, our ferrofluids present many advantages. They are biodegradable and non-toxic as they have been used for MRI contrast. As above, stirring is not required for the reaction. Because of the ferrofluid size, microscopy on recovered cells can be done without nanoparticle interference. Ferrofluid-labeled cells can be separated from open vessels (test tubes, chambers, blood bags) with external field magnets. Alternatively, they can be separated on high gradient magnetic columns that can readily be constructed or of the type used in other magnetic cell separation systems. For the latter separation method, cells have been separated with as few as three or four nanoparticles per cell.

For our clinical scale separator, X-GRAFFE™, ferrofluid-labeled cells are never suspended after the initial separation for the removal of bystander entrained cells, as is typically done with other magnetic beads. For that device, we have developed a method called “meniscus scrubbing“ that removes bystander cells from captured cells that are magnetically held in monolayers. That process has significant advantages which are (1) because cells are never cyclically suspended and separated very high yields are obtained, (2) because the method is so gentle, there are no effects on cell viability, and (3) efficiency of the process results in very high purities. The development of a system that incorporates those principles for the separation of smaller volumes such as cord blood and large research applications is in progress.

**Ferrofluid Products:**

BioMagnetic Solutions currently provides positive and negative cell selection kits, with ferrofluid and appropriate antibodies, as well as common capture ferrofluid reagents that can be paired with the antibody of your choice or used for other unique applications. The ability to create your own specific separation systems can be significantly advantageous and economical.

BioMagnetic Solutions is committed to advancing magnetic separations and expanding the applications of ferrofluids to advance your research efforts. Please contact us if you are interested in a product or application that we do not currently offer.

[1] K.C. Andree, G. van Dalum and L.W. Terstappen CellSearch Review. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5528971/pdf/MOL2-10-395.pdf

[2] This technology lead to the development of an immune-diagnostic platform for Eastman Kodak by our founder’s first company, Immunicon.