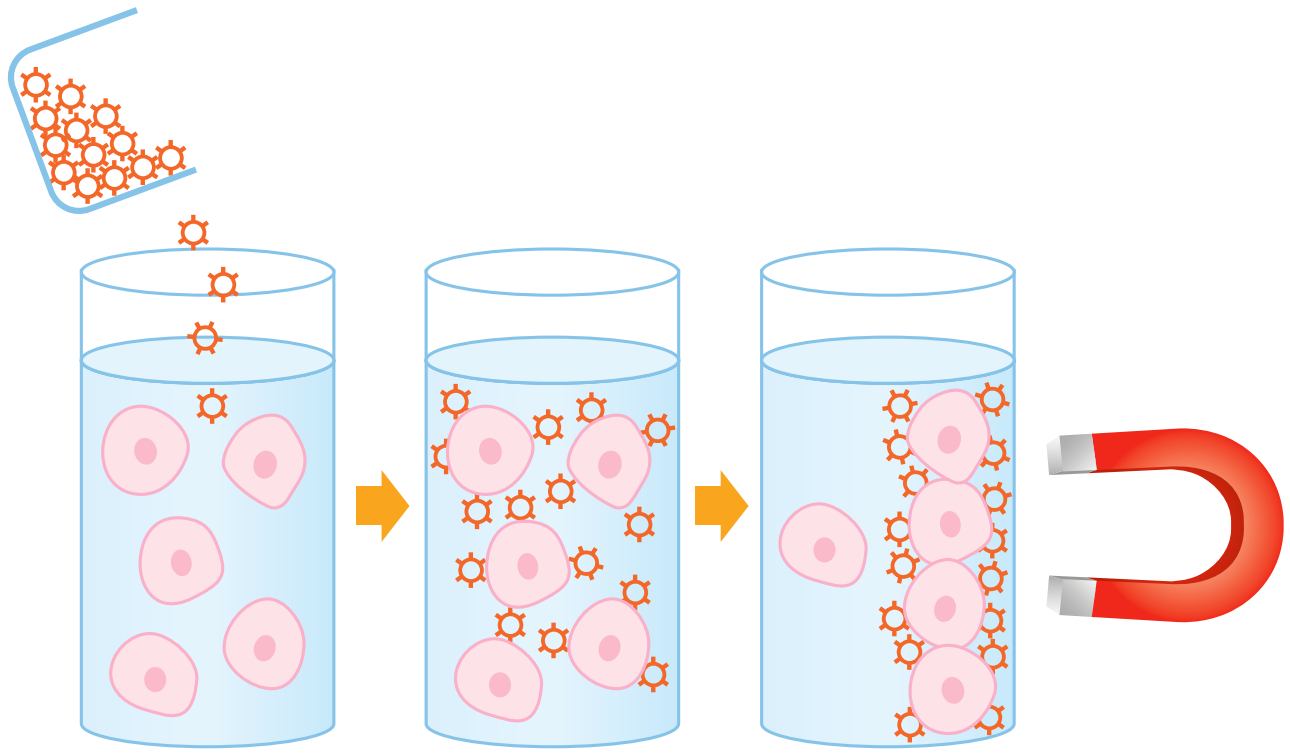
Does it matter which magnetic separation rack I use in my cell sorting processes?



Sepmag®

Does it matter which magnetic separation rack I use in my cell sorting processes?

Biomagnetic cell separation is an alternative to centrifugation, columns, filtration and precipitation. It eliminates undue cell-stress and reduces the risk of negative impact on cell function and phenotype.

However, in spite of the numerous types of magnetic beads available, the process does not always work as well as it should. Recovery is poor, cells are crushed by excessive force, reproducibility is questionable and scale-up over few milliliters seems impossible.

In a typical process, magnetic beads are added to a cell-sample, which is then incubated. The magnetic beads then attach to cells via antibodies, pectin or other substances. If the right biomarker is selected, only the desired cells are labeled. When these labeled cells are placed with the entire mixed-cell population into a Biomagnetic Separation system, the targeted cells are pulled by magnetic force, separating them from the cell culture with the attached beads.

Selection can be positive by labeling the cells targeted for analysis or culture. Unlabeled cells are then pipetted out and discarded. Alternatively, negative selection labels unwanted cells which are left in the retention device and the supernatant is extracted without them.

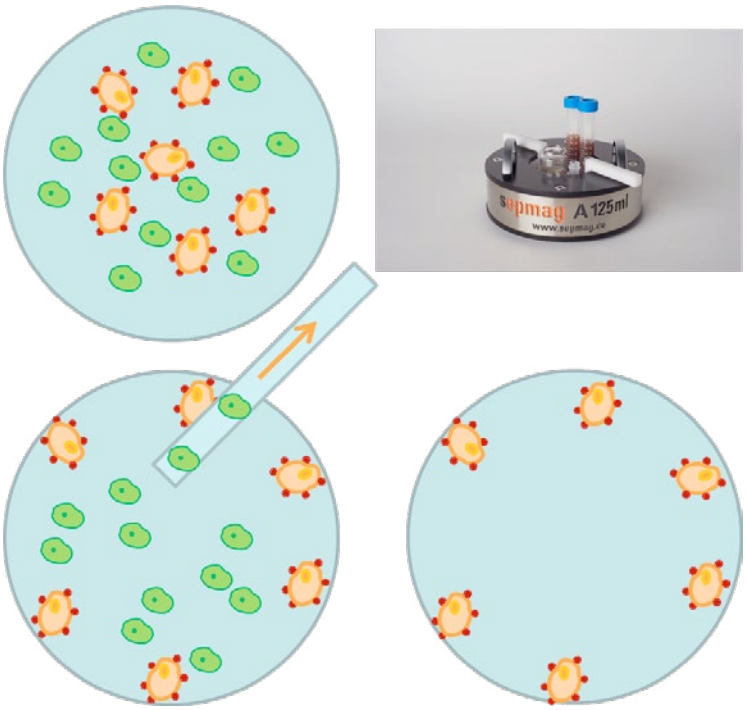


Figure 1. *Schematic representation of magnetic cell sorting process. Magnetic beads are added to a cell-sample, which is incubated (top left). Mixed-cell population is placed into a Biomagnetic Separation system, where targeted cells are pulled by magnetic force, separating cells with attached beads from the cell culture (bottom left). Non-labelled cells are extracted with the remaining supernatant. Only the labelled cells are retained (bottom right).*

Classical magnetic separation racks

Classical magnetic separation racks generate a non-homogenous magnetic force. The force decreases when farther away from the retention area, leading to longer separation times and/or cell losses. However, the separation force in the retention area is stronger, with a high risk of cell damage and reduced viability of the selected population (critical in positive selection) and the release of unlabeled remains to the supernatant (very significant in negative selection). Moreover, different forces that depend on the position of the beads cause cell shearing, with consequential risk of lysis during the process.

The first problem, cell loss, it is difficult to distinguish from labelling issues. Users' first reaction is always to blame the magnetic beads, start looking for new suppliers and re-start the labelling protocols. However, in most of the cases the real problem lies with the separation rack. Switching beads does not solve the problem, failure to achieve positive results leads to frustration, and users lose interest in magnetic cell sorting.

The inhomogeneous nature of the force also suggests that although separation may sometimes work well in the initial scale, as soon as a different well or tube volume is used, the magnetic force experienced by the cells is entirely different. Even if the issues do not appear in the initial scale, they may well do so when the project volume (and investment) increases, putting a lot of pressure on the development and production teams.

To avoid these problems it is necessary to take a closer look on how magnetic separation racks work.

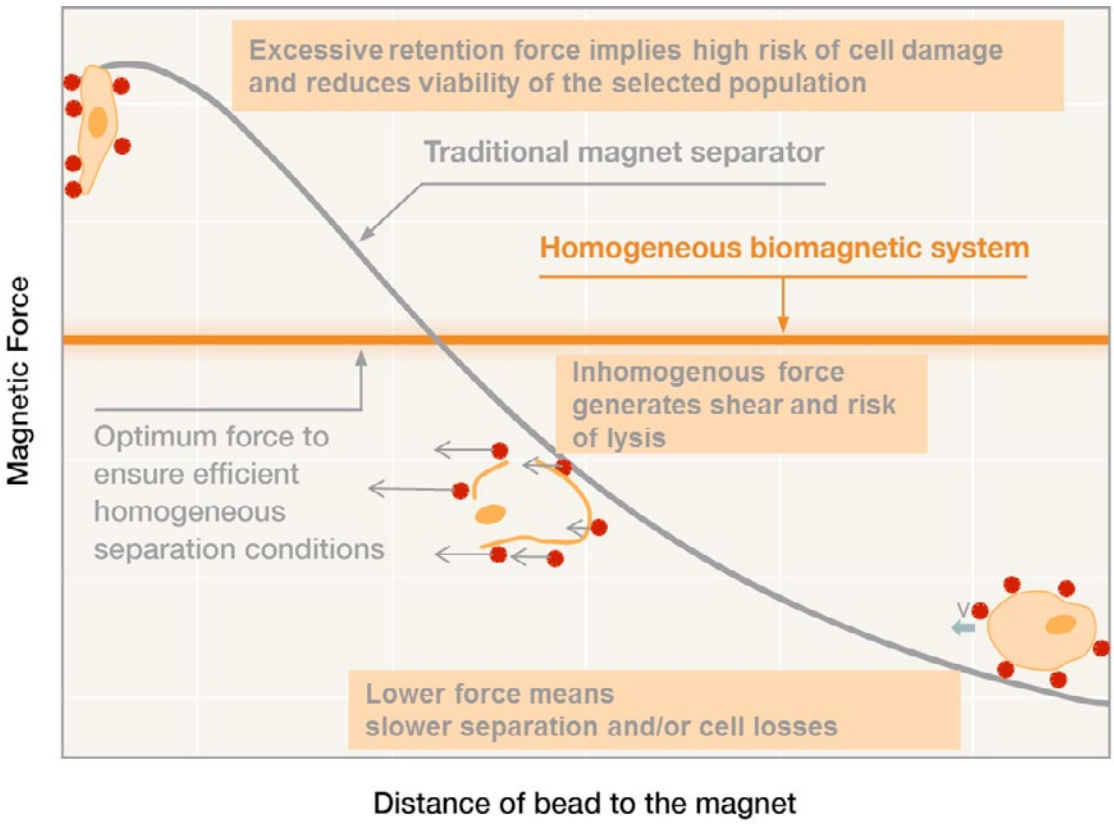


Figure 2. *Effect of the inhomogeneity of the magnetic force over the cells labelled with magnetic beads.*

Magnetic force and magnetic field are not equivalent

Magnetic force is related to the magnetic moment of the beads attached to the cell and magnetic field variation.

The value of the magnetic field (how many Tesla or Gauss you measure) is irrelevant. A uniform magnetic field does not generate a magnetic force. The force is directly proportional to the magnetic field gradient (when the magnetic beads are saturated) or to the gradient of the square of the magnetic field (when the field is very small). Put simply, the magnet generates a magnetic force over the magnetic beads, not because it generates a strong magnetic field, but because it generates a magnetic field that varies quickly with distance.

For a single permanent magnet (and classical magnetic separation racks), the variation of the magnetic field generates a force that also varies with distance, however, the situation is very different with modern Biomagnetic Separation Systems.

Modern homogenous Biomagnetic Separation systems

The last ten years has seen rapid growth in CLIA-kits and the IVD industry has faced similar problems on a larger scale.

To meet increased demand, R&D and Production departments want biomagnetic separation equipment that will enable them to be more efficient, validate processes, and which can also be scaled up (to tens and liters) and down (to microliters).

This demand has led to the development of homogenous biomagnetic separation systems. With special magnetic field profiles, these devices generate the right conditions (magnetic field and magnetic field gradient) for applying homogenous magnetic force to the entire working volume.

That means a stronger force farther from the retention area than that exerted by classical magnetic separators. The cell sorting process is therefore quicker and cell recovery rates are higher.

However, these modern systems have a gentler retention force, allowing higher viability of the selected population.

The homogenous value of the force means that beads experience the same force throughout the process, with no stress on the labelled-cell membranes.
Well-defined homogenous force value means that separation conditions are easy to replicate at different volumes, from ml to tens of liters. Even when small tubes are introduced in a large volume, the magnetic force experienced by the labelled cells remains the same.

Last, but not least, as the force is well defined (the value is constant throughout the working volume), different values can be tested to optimize conditions: larger for faster separation or lower for gentler conditions.

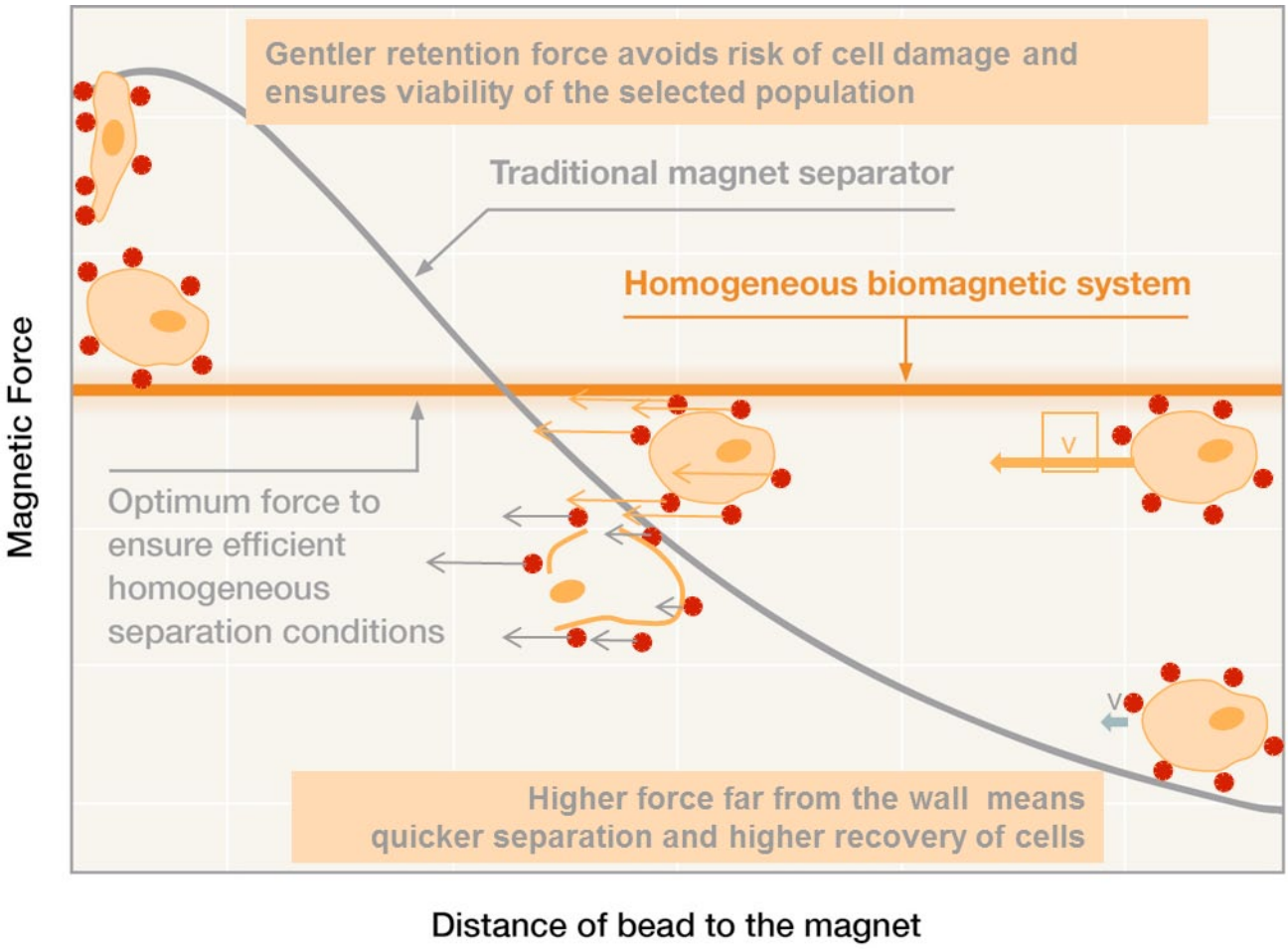


Figure 3. *Comparison between homogenous and inhomogeneous magnetic force effect over the cells with magnetic beads attached*

CONCLUSION

Growing demand for cell sorting protocols is putting a lot of pressure on development. Great efforts are made to select the right markers and magnetic beads to ensure their correct attachment to the desired cells.

However, all this effort goes to waste if the wrong magnetic separation rack is used. With inhomogeneous magnetic force is difficult to distinguish when cell sorting problems are caused by the biomarker or the separation process itself. Problems may occur in solutions developed at sub-milliliters when these are scaled up.

To ensure optimal performance of the magnetic beads selected to capture and isolate cells, the biomagnetic separation systems used should have a homogenous magnetic force. The value of the force should be carefully selected to ensure high recovery rates and gentle retention.

ABOUT THE AUTHOR

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Founder of SEPMAG®, Lluis holds a PhD in Magnetic Materials by the UAB. He has conducted research in German and Spanish academic institutions. Having worked in companies in Ireland, USA and Spain, he cumulates more than 20 years of experience, applying magnetic materials and sensors to industrial products and processes. He has filed several international patents on the field and co-authored more than 20 scientific papers, most of them related with the movement of magnetic particles. martinez@sepmag.eu

Learn the latest on magnetic bead cell separation

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