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Stem cell separation through cryogel

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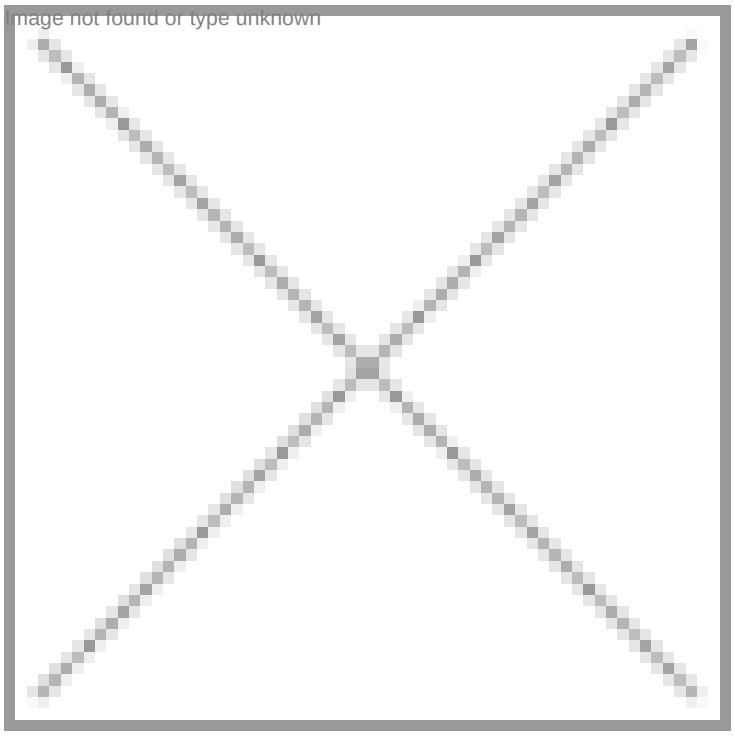
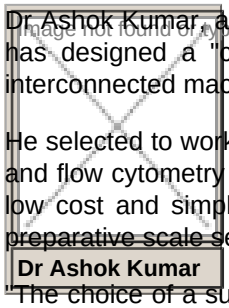


A new affinity chromatography column has been developed for cell separation systems.

With the advent of new sciences and technologies, there has been a need for developing new polymers, materials and techniques to put the novel knowledge into application. For instance, both in the areas of biomedical research and diagnostic medicine, it is vital to have specific separation of a discrete population of cells from a mixture. Through cell separation applications like fluorescence-assisted cell sorting via flow cytometry; density-gradient-based methods and magnetic-particle-based methods, a particular type of cell can be isolated from a complex mixture. The isolation of specific cell sub-populations is a key factor to the advancement of cell-based therapies of cancer, auto-immune diseases and genetic disorders.



Dr Ashok Kumar

Dr Ashok Kumar, associate professor, department of biological sciences and bioengineering, IIT Kanpur, along with his team has designed a "cryogel" that is, a polymeric gel formed in moderately frozen media, having a continuous system of interconnected macropores which is capable of separating stem cells.

He selected to work on developing a novel affinity chromatography column (the cryogel) because though magnetic separation and flow cytometry represent the most powerful tools for cell separations, they are limited to analytical applications. Owing to low cost and simple operation, cell affinity chromatography is considered the preferred approach when the application is preparative scale separation.

"The choice of a suitable matrix material is important because, as separation objects, cells are relatively large and are rather fragile and sensitive to shear stress. Their diffusivity is negligible and only convective transport can be used. Thus, for cell

affinity chromatography the key element is the design of the matrix", said Dr Kumar.

Cryogel as matrix

The cryogels are essentially gel matrices that are formed in moderately frozen solutions of monomeric or polymeric precursors. They typically have interconnected macropores or supermacropores with a size of 10-100 µm, allowing unhindered diffusion of solutes of practically any size, as well as mass transport of nano and even microparticles. The unique structure of cryogels, in combination with their osmotic, chemical and mechanical stability, makes them attractive matrices for chromatography of biological nanoparticles (plasmids, viruses, cell organelles) and even whole cells.

Model for stem cell separation

To demonstrate that stem cell separation can take place through the cryogel column, the researchers selected the human acute myeloid leukemia cells having CD34+ cell surface receptor. After labeling these CD34+ cells with anti CD34+ monoclonal antibodies, labeled cells were found specifically bound to Protein A-carrying supermacroporous monolithic column when passed through it. The viable CD34+ cells were later eluted when free non-specific cheap antibodies displaced cell-bound specific antibodies.

The CD34+ surface antigen is recognized as an important marker for hematopoietic stem cells. Thus, the scientists concluded that the system could be a good model for the separation of CD34+ cells from bone marrow or peripheral blood. Earlier, the researchers also demonstrated specific fractionation of T- and B-lymphocytes from human blood using antibodies against surface receptors of B-cells.

Benefitsa

In addition to a simple and elegant model for stem cell separation, the cryogel allows maximum recovery as it is a hydrophilic polymer on which the cells do not adsorb non-specifically. The other significant advantage is that there is no need for any approval from the regulatory authorities, since they are produced from exactly the same polymers as used in traditional chromatographic materials, which are already approved by regulators. All the synthetic chemistry used at present to prepare chromatographic materials is also applicable to cryogels. It has no other added chemicals and only ice crystals serve as porogen. The same standard low-pressure chromatographic systems can be used with cryogels.

Elaborating further about its advantages, Dr Kumar said, "Cryogels are extremely easy to handle and put in a column. They can be dried and re-swollen directly in the column. Due to the high polymer concentration in the walls of large interconnected pores, they are elastic. The monolith is easily removed and placed back in the column with no leakage in between the monolith and the column walls. Thus, there is no by-pass and the elastic gel monolith sits tightly in the column". The cryogel can also be produced in different sizes and formats (rods, sheets, discs, microtiter plates, etc) with different pore sizes (0.1-100 µm).

Lastly, as compared to magnetic beads they can be applied for both positive and negative selection of viable cells. There is also an efficient and economic use of the expensive monoclonal antibodies as no chemical attachment or immobilization of monoclonal antibodies is required. And there is no restructuring of the column required as the same column type is used for the separation of different cells, besides the ease and simplicity of operation.

"At present we are now both expanding the application of the affinity cryogel adsorbents to other cell separation processes and also simultaneously linking up with biomedical research laboratories and industries for further evaluation of gels on stem cell separation systems," said Dr Kumar.n