BioSpectrum the business of Bio & Health Sciences

Cryogel simplifies cell separation

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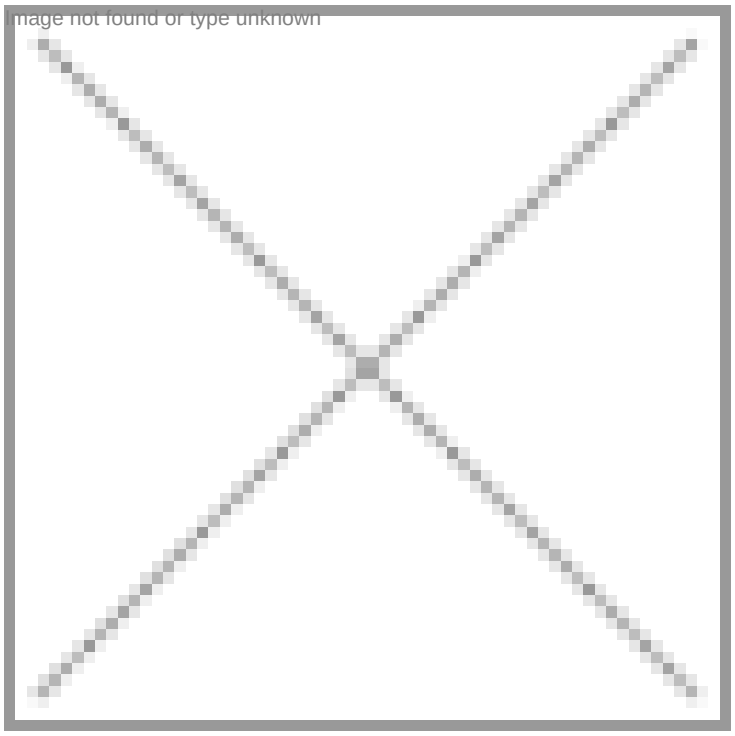
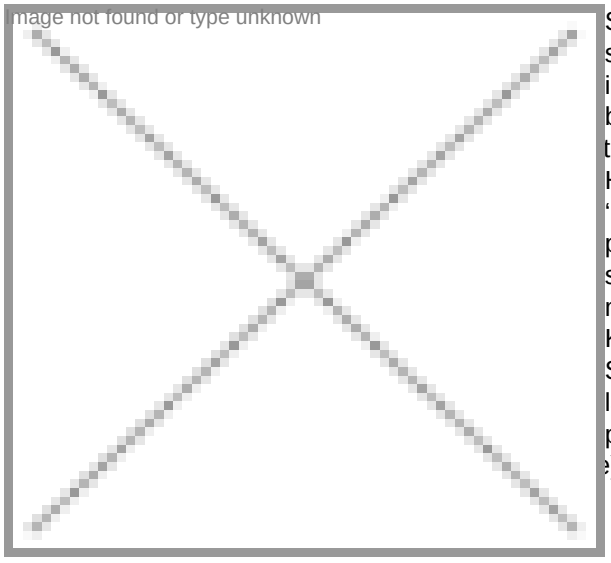
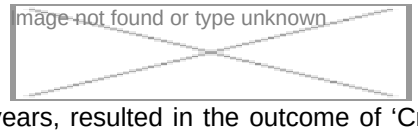


The unique 'Cryogel' matrix, invented by Dr Ashok Kumar and his team at IIT Kanpur, offers new possibilities using affinity cell separation for bioengineering applications. A follow-up report on the inital research reported in BioSpectrum, May 2005 issue



Separation or isolation of highly purified therapeutic mammalian cells such as stem cells, leukocytes at preparative scale, holds great importance in medical therapy and diagnostic applications. The bottleneck of currently available methods is their low yields, purity and t the analytical scale.

However, now an interesting and unique alternative, in the form of 'Cryogel' has come up in cell separation techniques. Cryogel is a polymeric gel formed in moderately frozen media, having a continuous system of interconnected macropores. This new macroporous 'Cryogel' matrix for bioengineering applications was developed by Dr Ashok Kumar, associate professor of bioengineering, Department of Biological Sciences and Bioengineering, IIT Kanpur, along with his team. Due to large interconnected pores, mechanically elastic properties and easy production, its application in cell separation has been enhanced, this was e).



The initial research in this area started in 2001; and in subsequent years, resulted in the outcome of 'Cryogel'. However, it

was yet to be established whether it can be used effieciently to separate all forms of cells in a real system. The project was supported by Department of Biotechnology (DBT) and Department of Science and Technology (DST), where the Cryogel was further used for more complex and a wide variety of cells. The work was further carried out to demonstrate the potential of affinity cryogel matrix for the separation of stem cells (CD34+), directly from umbilical cord blood and fractionation of B and T lymphocytes from peripheral blood. The DBT funded the project with 1 crore and the DST funding was around 30 lakh.

This work of Dr Ashok Kumar and Dr Akshay Srivastava has been recently published in Nature Protocols. Cryogel affinity matrix has been introduced as a new method for affinity cell separation.

Dr Ashok Kumar who received his PhD in biotechnology in 1994, jointly from Institute of Genomics and Integrative biology (IGIB), New Delhi and Indian Institute of Technology (IIT), Roorkee, has been working in this area, for more than a decade. After finishing his post-doctoral research at Nagoya University, Japan and Lund University, Sweden, he also worked as faculty of biotechnology in Lund University, Sweden and a group leader in a biotechnology company in Sweden.

Dr Kumar is also on the executive board of biomaterials and tissue engineering research and teaching in India; and servesas a task force committee member of DBT.

At the international level, besides conducting active international research collaborations with the UK, the US, Japan and Sweden, he is the co-coordinator for Indo-US center for Biomaterials supported by India-US Science and Technology Forum. He is incharge of the India-UK, DST-UKIERI award project and the India-UK science bridge project.

Unique Methodology

Dr Kumar, whose research areas have been in advanced biomaterials, stem cells, regenerative medicine, tissueengineering, bioprocess engineering and environmental biotechnology, has created a unique methodology to seperate the cells.

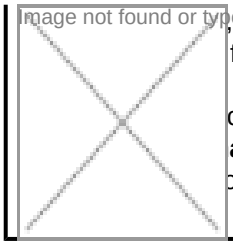
Commenting on the methodology used, Dr Kumar says, “Monolithic polyacrylamide and polydimethylacrylamide cryogel matrices were prepared using cryogelation technology. The supermacroporous monolithic cryogel has large interconnected pores (up to 100 ?m) that help in convective migration of mammalian cells. These cryogel matrices are further functionalized to immobilize a protein A ligand, by a simple and elegant chemistry. Stem cells or lymphocytes were labelled with specific antibodies; and then separated from other cell populations, while passing through cryogel affinity matrix. These cells were isolated with high cell recovery yield, while retaining their viability.”

The spongy and elastic character of cryogel matrix has provided a generic way for cell release from cryogel affinity matrix, by compressing the cryogel upto 50 percent of its original length and release of capillary liquid; called mechanical squeezing. The overall cell separation process takes less than 30 minutes for complete separation of cells on affinity cryogel matrices. The 100ml to 200ml of blood can be passed through the column using the cryogel matrix, which is not possible in case of other available columns for cell separation.

Besides being actively involved in research focused on cell separation technology and leukocyte filtration devices, Dr Kumar has done some innovative work in regenerative medicine and environmental biotechnology. The major research achievements of Dr Kumar in regenerative medicine include designing of neo-cartilage and bioartificial lever support bioreactor, using cryogel matrices that are at the preclinical stages. In the area of environmental biotechnology, Dr Kumar has suitably designed membrane filtration devices for air and water purification, like arsenic and microbial removal from drinking water and tar removal from cigarette smoke.

Technology Advantage

Cell separation using cryogel has many advantages over existing cell sorting techniques. Cryogel-based cellchromatography process could be an ideal separation system which can maintain sterility during the whole process. Overall separation strategy is generic, and the same cryogel column can be used for separation of multiple cell-types, up to four-to-five columns of regeneration. It is easy to execute chromatography using cryogel at any place, such as inside sterile laminar flow, besides in normal laboratory conditions. And the set-up can be moved from one place to another, without much trouble. The cytometry machine which costs about 2 crore is not available to every reseracher easily. Therefore, Cryogel offers access to efficient cell separation at a low cost.



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Dr Kumar who was part of the Center for Bioseparation in Sweden during 2001-2004 observes, “By using cryogel, the target cells could be separated at a preparative scale for medical application. Large volumes of the sample can be processed at once, through affinity cryogel matrix, which saves overall separation time, in comparison to other methods.” On cost effectiveness of the technique he says, “Target cells bind to affinity cryogel matrix, then these target cells are recovered in the generic way of elution called mechanical squeezing, that saves time and the process becomes cost-effective. Hence, cryogel-based cell affinity chromatography provides a generic way of cell separation.”

Besides being a researcher, Dr Kumar has published over 100 peer reviewed research papers in international journals, and has written more than 10 book chapters; and has several patents in his area of research. He is the editor of three books on cell separations and biomaterials. He is the associate editor of Nanoscale Research Letters; and is on the editorial board of several other biological and biotechnological journals. He is the executive board member of the
Federation of Asian Biotechnology.

Industry Benefits

The cryogel support as a chromatography matrix could have a huge impact on research and development at laboratory as well as industrial scale. The developed method using cryogel is easy and cost-effective. It also eliminates steps of pre-purification of sample during separation. The target cell could be separated in a single step, with sufficiently high purity and yield, which is quite useful for the preparation of target cells in large concentrations; and yields for its application in cell therapy, tissue engineering, cell-based studies. This first time approach of using no buffer or effulent, and reusage of matrix, can cost approxmately about 500, which is far cheaper than any other alternatives.

Dr Kumar is optimistic about the transfer of this technogy to industry; and expects to be approached by companies verysoon. In fact, he has been in talks with some companies for the same. On commercialization of the technology, Dr Kumar says that, “The technology is ready-to-use, and further contacts with industries are being established that will help to take this stem cell separation technology to commercialization.”

The transfer of this home-grown technology to industry will surely bring down the cost of cell separation, besides making it easier for researchers to avail benefits that come with it.

Rahul Koul in New Delhi