Bio-Medical

Hydrocyclone/Filter for Concentrating Biomarkers From Soil

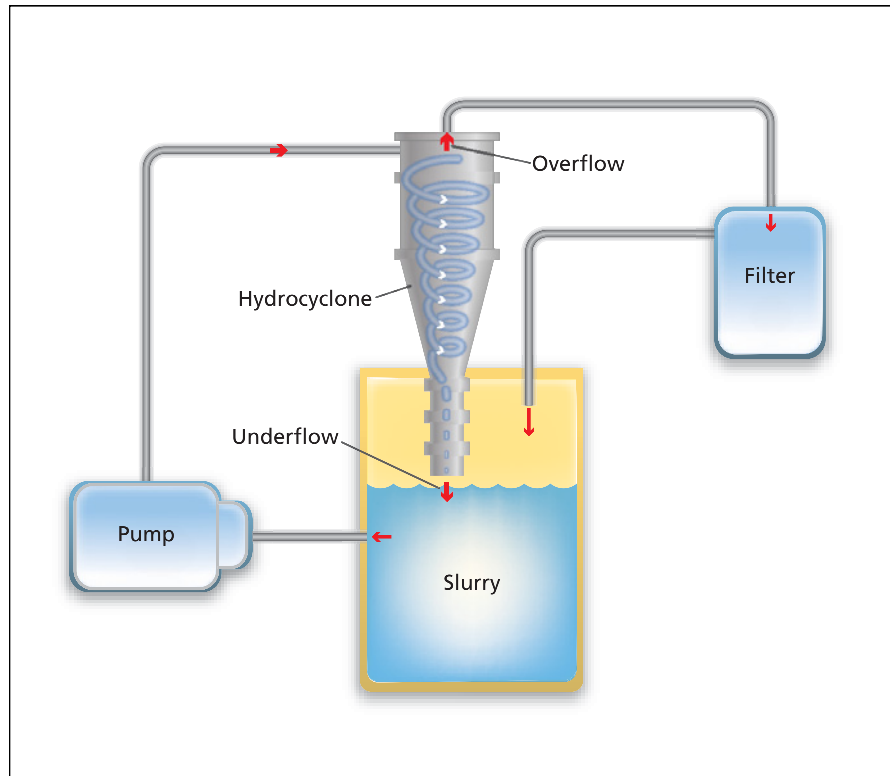
This apparatus could detect biomarkers or chemical waste in soil.

NASA's Jet Propulsion Laboratory, Pasadena, California

The hydrocyclone-filtration extractor (HFE), now undergoing development, is a simple, robust apparatus for processing large amounts of soil to extract trace amounts of microorganisms, soluble organic compounds, and other biomarkers from soil and to concentrate the extracts in amounts sufficient to enable such traditional assays as cell culturing, deoxyribonucleic acid (DNA) analysis, and isotope analysis. Originally intended for incorporation into a suite of instruments for detecting signs of life on Mars, the HFE could also be used on Earth for similar purposes, including detecting trace amounts of biomarkers or chemical wastes in soils.

In addition to a conical separator vessel typical of a hydrocyclone, the HFE includes a pump, a sample container, an electropositive nanoparticle filter, and associated plumbing (see figure). The hydrocyclone serves to separate both cellular-sized particles (<5 μm) and dissolved organic compounds from the bulk soil (consisting mostly of particles larger than 5 μm). The electropositive filter serves to capture cellular biomass and remove particles from the extract with maximal capacity (that is, minimal clogging).

The soil sample is prepared by mixing it with sterile water or another suitable solvent to form a slurry, which is pumped to the hydrocyclone separator vessel in tangential streams at the top (wide) end to form two concentric opposing vortices at high hydrodynamic pressures to effect separation based on particle size. The spinning motion of the injected slurry is accelerated by the conical taper, creating a large centrifugal force that causes the larger particles to rapidly separate from the rest of the flow and leave the vessel as part of underflow through the bottom (narrow) end of the separator vessel. The liquid containing suspended particles smaller than 5 μm leaves the vessel as the overflow (which is larger than the underflow) at the top (wide) end of the vessel. The overflow is then fed through the filter.



In this **Prototype HFE**, a soil/water slurry is pumped from a sample container through a hydrocyclone separator vessel and a filter and is recycled through the sample container. The hydrocyclone separates most of the soil particles into an underflow. The filter traps smaller particles in the overflow.

The filter contains electropositive aluminum nanotubes embedded in a glass-fiber matrix. The filter has a nominal pore size of 2 μm, but can collect biological particles having sizes down to fractions of a micron, on the basis of electrostatic charge, without clogging. The combination of the hydrocyclone and the electropositive nanoparticle filter is capable of extracting 99 percent of particles smaller than 5 μm from the soil sample and retaining them for analysis. In the prototype HFE depicted schematically in the filter, the underflow and the effluent from the filter are fed back to the sample container. It is envisioned that the fully developed HFE could operate in a continuous-flow mode, making it possible to extract biomarkers from a large volume (e.g., 1 m^3) of soil using a minimal amount (e.g., 1 L) of solvent.

In a field test, the prototype HFE was made to process a slurry consisting of 4 L of sterile water mixed with 4 L of Atacama-desert soil, which contains such low concentrations of recoverable microorganisms as to be nearly impossible to analyze in the absence of an extraction-and-concentration process. After 15 minutes of recycling the slurry in the HFE, the maximum loading of the filter was reached. The filter was removed and dried and found to contain 100 g of fine particulate material, which, in turn, was removed from the filter in a laboratory for further analysis. Whereas traditional extraction protocols yielded no microbial colony-forming units, the extract from the HFE filter yielded microbes too numerous to count on standard growth plates. On the basis of development plans and this result, it is expected that

cellular-extract concentrations obtained by use of the fully developed HFE will be at least 10^3 times those obtained by means of state-of-the art extraction methods heretofore used in environmental microbiology.

This work was done by Adrian Ponce of Caltech and Donald Obenhuber of National Research Council for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1).

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

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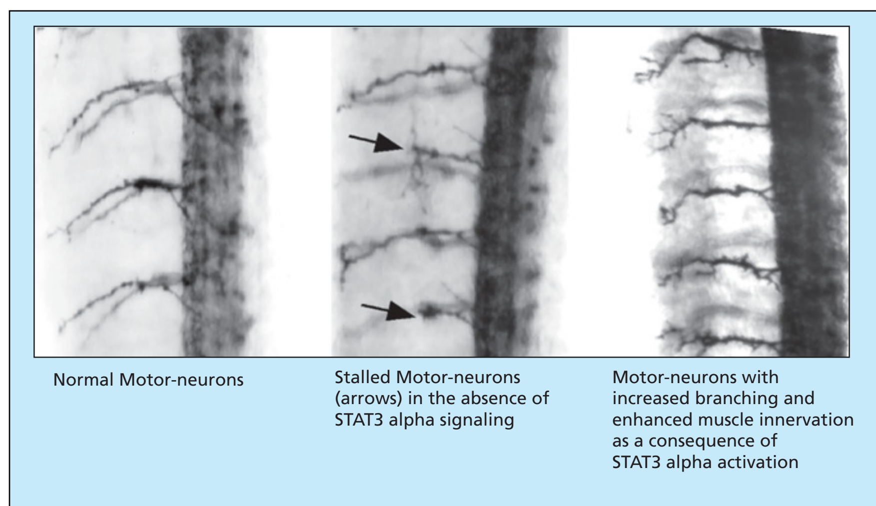
Refer to NPO-44751, volume and number of this NASA Tech Briefs *issue, and the page number.*

Activating STAT3 Alpha for Promoting Healing of Neurons

Natural anti-apoptotic, pro-axogenic mechanisms are stimulated artificially.

Ames Research Center, Moffett Field, California

A method of promoting healing of injured or diseased neurons involves pharmacological activation of the STAT3 alpha protein. Usually, injured or diseased neurons heal incompletely or not at all for two reasons: (1) they are susceptible to apoptosis (cell death); and (2) they fail to engage in axogenesis — that is, they fail to re-extend their axons to their original targets (e.g., muscles or other neurons) because of insufficiency of compounds, denoted neurotrophic factors, needed to stimulate such extension. The present method (see figure) of treatment takes advantage of prior research findings to the effect that the STAT3 alpha protein has anti-apoptotic and pro-axogenic properties.



Motor Neurons visualized by a dark staining reaction are seen exiting the spinal cord, from the right side of each panel, innervating adjacent muscle tissue.

As used here, "STAT" signifies "signal transducer and activator of transcription." "STAT3 alpha" is the name of one of a number of transcription factors and of the gene responsible for producing it. Transcription factors activate the expression of other genes and otherwise generally regulate gene expression. The STAT3 alpha protein is activated in response to such extracellular factors as hormones and growth factors as well as the aforementioned neurotrophic factors. The STAT3 alpha protein associates with trans-membrane receptors for these extracellular factors. When activated by growth factors and hormones, STAT3 alpha prevents apoptosis.

Recent findings from the Life Sciences and Microgravity Division of Ames Research Center now show that when activated by neurotrophic factors STAT3 alpha, in addition to preventing apoptosis, also promotes innervation. The natural activation of the STAT3 alpha protein is effected by phosphorylation of a specific tyrosine amino acid. Upon such phosphorylation, two STAT3 alpha molecules can form a homo-dimer. The tyrosine on a given STAT3 protein molecule that becomes phosphorylated resides on a flexible activation loop sequence that binds to a pocket, denoted the SH2 domain, of another STAT3 protein molecule. Upon homo-dimerization, STAT3 alpha is retained in the nucleus of cells, where it binds specific deoxyribonucleic acid (DNA) sequences and activates the expression of nearby genes. Thus, homo-dimerized STAT3 alpha protein is part of a signal-transduction complex that relays chemical signals from hormones, growth factors, and neurotrophic factors outside the cell directly to the DNA inside the cell. In so doing, it activates genes that exert anti-apoptotic and axogenic effects.

Hence, the present method is based on the design of a new class of pharmacological agents (homo and heteroditopic Janus molecules) to promote artificial dimerization and pharmacological activation of therapeutic targets, in this case the STAT3 alpha protein. The precise location of each atom in the STAT3 homodimer is known and it is possible to use this structural information along with molecular modeling and docking programs to rationally design a Janus molecule having the correct sizes and shape, and/or to bind to STAT3 alpha preferentially to other proteins that are similar to STAT3 alpha. The compound can be synthesized by techniques that are well established in the pharmaceutical industry and can be formulated to be administered alone or in combination with other therapeutic compounds.

This work was done by Greg Conway of Ames Research Center.

This invention is owned by NASA, and a patent application has been filed. Inquiries concerning rights for the commercial use of this invention should be addressed to the Ames Technology Partnerships Division at (650) 604-2954. Refer to ARC-15475-1.