hour incubations and 75 percent for 3-hour incubations. Interestingly, samples incubated for less time (2 hours vs 3 hours) produced an increased percentage of antigen detection. Further testing at incubation times such as 1 hour or lower could potentially increase positive predictability based on the study's results. Also encouraging were negative control experiments with nonspecific antigens, beta galactosidase and thyroglobulin, which showed results of 100 percent accuracy, with no false positive detection.

*This work was done by Maximilian C. Scardelletti and Vanessa Varaljay of Glenn Research Center. Further information is contained in a TSP (see page 1).*

*Inquiries concerning rights for the commercial use of this invention should be addressed to NASA Glenn Research Center, Innovative Partnerships Office, Attn: Steve Fedor, Mail Stop 4–8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-18387-1.*

# Isolation of Precursor Cells From Waste Solid Fat Tissue

*Lyndon B. Johnson Space Center, Houston, Texas*

A process for isolating tissue-specific progenitor cells exploits solid fat tissue obtained as waste from such elective surgical procedures as abdominoplasties ("tummy tucks") and breast reductions. Until now, a painful and risky process of aspiration of bone marrow has been used to obtain a limited number of tissue-specific progenitor cells.

The present process yields more tissue-specific progenitor cells and involves much less pain and risk for the patient. This process includes separation of fat from skin, mincing of the fat into small pieces, and forcing a fat saline mixture through a sieve. The mixture is then digested with collagenase type I in an incubator. After centrifugation tissue-specific progenitor cells are recovered and placed in a tissue-culture medium in flasks or Petri dishes. The tissue-specific progenitor cells can be used for such purposes as (1) generating three-dimensional tissue equivalent models for studying bone loss and muscle atrophy (among other deficiencies) and, ultimately, (2) generating replacements for tissues lost by the fat donor because of injury or disease.

*This work was done by Diane Byerly of Johnson Space Center and Marguerite A. Sognier of Universities Space Research Association. Further information is contained in a TSP (see page 1). MSC-23775-1*

# Identification of Bacteria and Determination of Biological Indicators

**Identifying mechanisms of micro-organisms can prevent forward contamination in space missions and can help in developing new antibiotics and amino acids.**

*NASA's Jet Propulsion Laboratory, Pasadena, California*

The ultimate goal of planetary protection research is to develop superior strategies for inactivating resistance-bearing micro-organisms like *Rummelibacillus stabekisii*. By first identifying the particular physiologic pathway and/or structural component of the cell/spore that affords it such elevated tolerance, eradication regimes can then be designed to target these resistance-conferring moieties without jeopardizing the structural integrity of spacecraft hardware. Furthermore, hospitals and government agencies frequently use biological indicators to ensure the efficacy of a wide range of sterilization processes. The spores of *Rummelibacillus stabekisii*, which are far more resistant to many of such perturbations, could likely serve as a more significant biological indicator for potential survival than those being used currently.

Numerous surveys of the contaminant microbial diversity housed within spacecraft assembly facilities over the past six years have resulted in the recurrent isolation of spore-forming bacteria belonging to the *Bacillus* genus. As *Bacillus* species are capable of existing as metabolically inactive, extremely hardy spores, many lineages exhibit remarkable resilience to varying modes of bioreduction/sterilization aimed at their eradication (UV and gamma radiation, oxidizing disinfectants, etc.). The microorganism *Rummelibacillus stabekisii sp. nov.* was isolated from the surfaces of the cleanroom facility in which the Mars Exploration Rovers (MER) underwent assembly. This bacterium has not been previously reported, and shows no close relation to any previously described species (as is assessed via 16S rRNA gene sequence comparison). This unique isolate, and the *Bacillus* species most genetically similar to it, were subjected to a multitude of biochemical tests in order to thoroughly characterize its taxonomic position based on physiological and phylogenetic evidence. The results clearly show that this bacterium is significantly different from its nearest relatives.

The microbial colonization of spacecraft and cleanroom assembly facility surfaces is of major concern to NASA and others commissioning modern-day space exploration. The search for life elsewhere in the solar system will rely heavily on validated cleaning and sterility methods. It would be devastating to the integrity of a mission directed at pristine environments such as the Europa's subsurface ocean or the Martian polar caps to be compromised as a result of terrestrial microbial contamination. To this end, planetary protection policies are in place to ensure the cleanliness and sterility of mission-critical spacecraft components in order to prevent forward or backward contamination.

Spores of *Bacillus subtilis*, a model spore-forming laboratory strain that demonstrates higher susceptibility to ultraviolet and gamma radiation than

other wild type spore formers, have nevertheless been shown to survive up to six years under interstellar space conditions. Previously undescribed spore-forming species, such as *Rummelibacillus stabekisii*, may exhibit even greater resilience. It is in the best interests of NASA to thoroughly understand the physiological capabilities of each and every novel micro-organism isolated from these spacecraft-associated cleanrooms.

*This work was done by Kasthuri Venkateswaran, Myron T. La Duc, and Parag A. Vaishampayan of Caltech for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov. NPO-46221.*

# Further Development of Scaffolds for Regeneration of Nerves

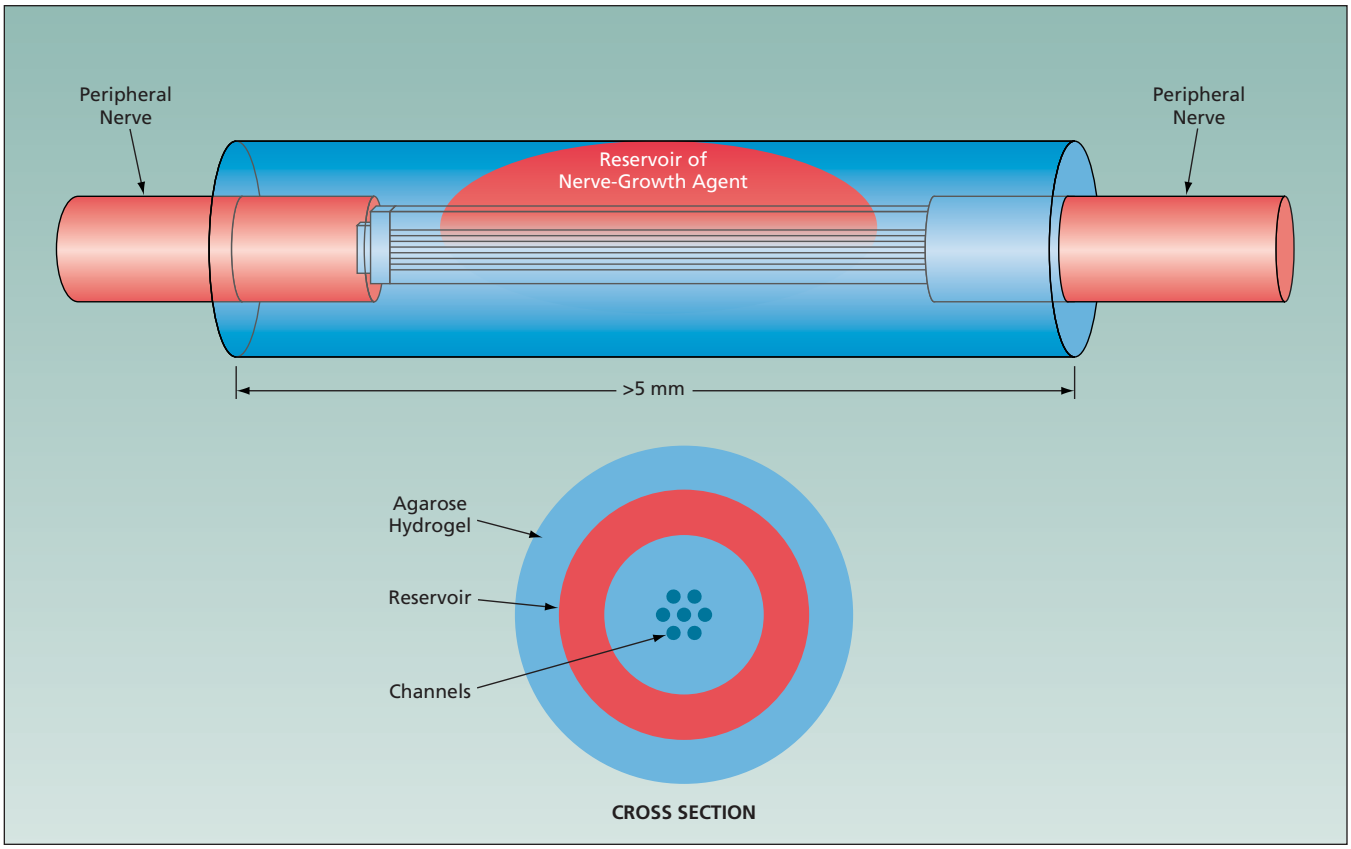
## Scale-up toward clinically significant dimensions has been partially completed.

*NASA's Jet Propulsion Laboratory, Pasadena, California*

Progress has been made in continuing research on scaffolds for the guided growth of nerves to replace damaged ones. The scaffolds contain pores that are approximately cylindrical and parallel, with nearly uniform widths ranging from tens to hundreds of microns. At the earlier stage of development, experimental scaffolds had been made from agarose hydrogel. Such a scaffold was made in a multistep process in which poly(methyl methacrylate) [PMMA] fibers were used as templates for the pores. The process included placement of a bundle of the PMMA fibers in a tube, filling the interstices in the tube with a hot agarose solution, cooling to turn the solution into a gel, and then immersion in acetone to dissolve the PMMA fibers. The scaffolds were typically limited to about 25 pores per scaffold, square cross sections of no more than about 1.5 by 1.5 mm, and lengths of no more than about 2 mm.

To be clinically relevant, the scaffolds must be scaled up: They are required to have typical cross-sectional dimensions of the order of 1 cm and to have lengths in the approximate range of 2 to 2.5 cm. For repairs of the central nervous system, there is an additional requirement that each scaffold contain between about 100 and about 1,000 pores; for repairs of peripheral nerves, there is a requirement for sustained or timed release of brain-derived neurotrophic factor (BDNF) or another suitable nerve-growth agent to enable growth to continue to the required lengths.

The work performed since the earlier stage has been oriented toward satisfying these and other requirements. The work has included development of a more-complex version of the prior multistep process that has made it possible to partly satisfy the scaling-up requirements in that scaffolds having cross sections exceeding 1 $cm^2$ in area and lengths up to 1 cm have been fabricated. One notable feature of the present version of the process is a multistep subprocess in which a template of polystyrene (PS) fibers is made from a composite of polystyrene fibers surrounded by a continuous PMMA matrix. Another notable fea-




A **Nerve-Growth Scaffold** containing a reservoir of a nerve-growth agent would be attached to severed ends of a peripheral nerve.