PHYSICOCHEMICAL PROCESSES IN BIOLOGICAL SYSTEMS IN SPACE

Final Report of the Overstudy Panel

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Executive Summary

Every biological process involves physicochemical processes. Under microgravity conditions, these processes often differ significantly in comparison to the same systems on earth. Previous reviews of NASA's research programs in biological and life sciences have emphasized the need to understand the fundamental mechanisms that underlie these alterations. Since gravity influences behavior in the fluid state, it follows that physicochemical transport phenomena play significant roles. Close collaboration between biological and physical scientists will enhance the degree of understanding of these mechanisms, their significance and their applications. ***In this respect NASA's unique facilities and extensive expertise in the behavior of physical systems in weightlessness can play a key role in facilitating such research.***

To explore possibilities for multidisciplinary research in NASA's biological and life sciences programs, a panel of researchers from the physical and biological sciences was convened under the auspices of the National Center for Microgravity Research and the NASA-Glenn Research Center. The panel members were generally familiar with the broad spectrum of NASA's programs, but they focused on areas where an integrated cross-disciplinary approach would take advantage of NASA's unique expertise and facilities. Thus gravity can be used as a controlled variable to advance research in NASA's space related activities including astronaut health, biological systems, and biology-based engineering systems. ***An important conclusion from the panel's deliberations is that engineers, physicists, chemists, and biological and life scientists should be involved in integrated, cross-disciplinary research that combines experiments, modeling, and computational simulation.***

The panel suggested several representative research areas to elucidate the effects of microgravity and physicochemical processes on biological processes. The recommended research areas are put in context by a consideration of the effects of microgravity and the space environment on biological processes and by a description of the multidisciplinary method.

EFFECTS OF MICROGRAVITY AND THE SPACE ENVIRONMENT ON BIOLOGICAL PROCESSES

Microgravity has direct effects on tissues and the musculoskeletal, cardiopulmonary, and vestibular systems. The biological effects of microgravity on biological systems at the cellular level, however, are indirect and mediated through the alteration of a complex variety of physicochemical influences, such as hemodynamic and hydrostatic pressures, fluid shear stresses, three-dimensional tissue stresses, tissue transport, and permeability. Cells in all biological systems sense these direct and indirect effects in a variety of ways, through, for example, membrane-bound receptors and ion channels, cell shape, and cytoskeletal and membrane structure changes. On the molecular scale, microgravity effects are manifested through changes in flow and pressure that alter gene transcription by a signal transduction cascade. Changes in fluid transport conditions in microgravity may also affect drug delivery and pharmacokinetics. Other space-related phenomena such as cosmic radiation, "g-jitter", and atmosphere quality may also affect biological systems.

MULTIDISCIPLINARY APPROACHES TO MECHANO-BIOLOGY

An effective multidisciplinary investigation of the effects of microgravity on biological systems will require integrating parallel efforts in experimentation, modeling, and computation. In this approach to *mechano-biology*, relatively idealized models are initially formulated to support a specific hypothesis. The research then proceeds to ground- and space-based experiments combined with more advanced modeling. The approach is hierarchical and requires contributions from biological- and life-scientists and physical- and engineering-scientists. In some areas of the biological and life sciences, much of this paradigm is already established, but in other areas, it is expected to break new ground. Furthermore, NASA's support of academic programs to educate multidisciplinary scientists and engineers is needed to pursue these kinds of investigations.

RECOMMENDED RESEARCH AREAS

The effects of microgravity are manifested on biological systems through alterations in small forces, changes in fluid pressure, and other physicochemical processes. ***Thus, the panel recommended that the responses of biological systems (cells, tissues, etc.) to mechanical perturbations originating from the environment should be a primary subject of NASA's research emphasis. The development of instrumentation to measure the hypothesized effects of microgravity is an integral part of the recommended research.*** Research in medical and biological technologies will also require new, compact, minimally-invasive "smart" medical devices and environmental monitoring sensors.

The panel identified a number of representative areas of research for which a better understanding of the role of physicochemical processes in microgravity will lead to significant advances. The suggested research areas were grouped under two topics: *Cellular Physiology and Molecular Biophysics* and *Medical and Biological Technology*. All of the areas are crucial to NASA's mission or influence living and research conditions in space. Brief summaries of the recommendations are given below.

Cellular Physiology and Molecular Biophysics

- **Cell response to the environment:** investigate how receptors within the membrane of the cell can respond to microgravity (e.g., changes in fluid pressure across the membrane), using model systems such as dissociated cultured neurons (or, alternatively, endothelium, bone or muscle cells).

- **Cell differentiation:** investigate if and how microgravity affects cell differentiation, using a model system such as adult stem cells to determine if they can be activated by specific stimuli in microgravity (e.g., tissue damage), maintain their populations, and differentiate into all possible mature cell phenotypes.

- **Cell development:** investigate how the change from normal gravity to microgravity influences the development of cytoskeletal structures, using model systems such as *Caenorhabditis elegans* and *Drosophila melanogaster*, and also, how microgravity alters the cell loading on plant structures (gene expression, phenotypes), using a model system such as *Arabidopsis*.

- **Cell structure:** investigate the loss of human bone mass in microgravity, using, for example, the anabolic responses of bone-forming cells (osteoblasts) as a model system.

- **Tissue/vascular remodeling:** investigate the effects of microgravity on the tissue-fluid balance at the macro- and micro-circulatory levels, using intravital microscopy of a model system living circulation.

- **Molecular conformation, order, and structure:** investigate the physical mechanisms of mechano-chemical transduction associated with changes in the conformation of cytoskeleton and extracellular matrix proteins, transmembrane proteins, G-protein-related phenomena, or alterations in membrane structure or fluidity.

- **Genomic research:** identify the network of genes and their products whose expression and function are affected by alterations in physical forces and physicochemical processes in microgravity, using model organisms with tractable genomes such as *Caenorhabditis elegans* and *Drosophila melanogaster*.

- **Instrumentation:** extend existing methods for molecular study such as BioMEMS, fluorescence resonant energy transfer (FRET) and atomic force microscopy (AFM), and develop new techniques for force application to, and molecular modification of, surfaces.

Medical and Biological Technology

- **Drug delivery:** develop understanding of how existing drug delivery methods are influenced by microgravity and develop new delivery methods where necessary.

- **Pharmacokinetics and Pharmacodynamics:** develop improved models of pharmacokinetics and optimal monitoring strategies applicable to microgravity.

- **Medical devices and technologies:** develop devices that are compact and minimally invasive to assess drug delivery and pharmacokinetics and to aid in predicting and preventing disease; microfluidics devices will be prominent in these developments.

- **Contained environmental health and safety:** develop "smart" sensors to monitor and regulate the environment of spacecraft to mitigate environmental hazards.

- **Bioinformatics:** develop a unified, web-based, searchable database for all information from spaceflight biological experiments and astronaut physiologic studies.

Introduction

Through its Office of Biological and Physical Research, the National Aeronautics and Space Administration is pursuing an extensive program of intramural and extramural research in biological and life sciences[1]. The research is aimed at understanding the roles that gravity and the space environment play in biological processes related to fundamental scientific issues and to the health and support of astronauts. The program has been reviewed and evaluated several times, and recommendations have been made for a variety of research areas ranging from cellular development to human adaptation in space to life-support systems[2,3,4,5]. All the previous reviews have emphasized the need to understand the fundamental processes that cause the observed alterations in biological systems in microgravity. To understand the fundamental mechanisms, however, requires an understanding of the physicochemical processes that are ubiquitous in almost every biological process, from molecular and sub-cellular scales to entire systems. On the molecular and intracellular scales, physicochemical processes are relevant, for example, to genetics through signaling pathways that transduce stimuli associated with gene activation. On a larger scale, they play a role in both macro- and micro-circulation in the cardiovascular and pulmonary systems.

Clearly, integrated multidisciplinary investigations are needed to link the fundamental physicochemical processes to the relevant microgravity biological phenomena in order to achieve the goals of understanding the mechanisms by which a space environment influences biological processes and how the processes can be controlled. Even though this need is clear, collaborations between physical and biological scientists are not widespread at present. In addition, physical and biological scientists generally take a somewhat different approach to their research. Typically, physical and engineering scientists conduct their research by deductive reasoning, formulating models (analytical, computational, or laboratory models) based on physicochemical first principles, enumerating the parameters that influence the outcome of the phenomena under study, and conducting experiments to measure the phenomena when one or more parameters are varied. Typically, biological and life scientists conduct their research by inductive reasoning, formulating a specific hypothesis, undertaking controlled experiments with a model or actual system, making observations and measurements, and using statistical methods to verify or disprove the hypothesis. This approach to biological research has been effective. However, observations made from experiments in an earth-based laboratory have been subject to the pervasive and unchanging influence of earth gravity, and, consequently, the direct and indirect effects of gravity that underlie the biological responses would likely not be discovered by this method. Biological and life sciences experiments conducted in space that proceed in this same way would probably be unsuccessful in uncovering the fundamental mechanisms, considering the practical limitations on resources and access to the space environment. Instead, space-based biological and life science research should proceed by a multidisciplinary approach based on a combination of the biological and physical science approaches.

To explore the issues associated with multidisciplinary research, a panel of researchers from the physical and biological sciences was convened under the auspices of the National Center for Microgravity Research and the NASA-Glenn Research Center. The panel was familiar with the broad spectrum of research problems recommended by the previous reviews of NASA's biological and life science research program, including:

- crucial issues in cell biology that are affected by the space environment,
- specific changes in biological phenomena caused by long exposure to microgravity,
- development of biology-inspired materials (e.g., tissue engineering) and advanced technologies made possible or enhanced by a space environment, and
- technology for space research equipment and life support systems.

From this broad spectrum, the panel focused on a subset of cross-disciplinary research programs that would take advantage of NASA's unique space facilities and expertise to utilize gravity as a controlled variable and that would significantly enhance the understanding of biological systems and the development of biology-based engineering systems. These programs would couple biological investigations to physicochemical processes. The panel's recommendations should help NASA to formulate a cross-disciplinary research program that will bring together engineering, physics, chemistry, mathematics, biology, computer science, and materials science.

The panel members and their areas of current research are:

Scott L. Diamond, Ph.D. (Professor, Chemical Engineering Department and Director of the Biotechnology Program, University of Pennsylvania); research interests include gene therapy, mechano-biology, cardiovascular biorheology, heterogeneous bioreaction systems, coupled reaction-transport systems, fluorescence spectroscopy, and imaging.

Dominique M. Durand, Ph.D. (Professor, Biomedical Engineering and Neurosciences and Director of the Neural Engineering Center, Case Western Reserve University); research interests include neural engineering, computational neuroscience, and electrophysiology.

John A. Frangos, Ph.D. (Professor, Bioengineering Department, University of California at San Diego); research interests include shear stress activation of endothelial membrane function, microgravity *in vitro* modeling of bone, flow effects, interstitial fluid flow in bone remodeling, and novel strategies for tridimensional *in vitro* induction.

Roger D. Kamm, Ph.D. (Professor, Mechanical Engineering Department, Massachusetts Institute of Technology); research interests include respiratory mechanics and fluid dynamics, cell mechanics and mechano-transduction, and cardiovascular fluid dynamics.

Herbert H. Lipowsky, Ph.D. (Professor and Chair, Bioengineering Department, Pennsylvania State University); research interests include application of engineering techniques to solve problems in the physiology of microvascular function in health and disease.

Andrew D. McCulloch, Ph.D., (Professor, Bioengineering Department, University of California at San Diego); research interests include cardiac biomechanics, biomechanics, mechano-biology and electrophysiology, cardiac tissue engineering and cell-matrix interactions, computational physiology, and genomics of heart disease.

Larry V. McIntire, Ph.D. (E. D. Butcher Professor and Chair, Bioengineering Department, Rice University); research interests include bioengineering of vascular biology, cellular engineering, tissue engineering, and tissue culture reactors.

Ronald J. Midura, Ph.D. (Biomedical Engineering Staff, Lerner Research Institute, The Cleveland Clinic Foundation); research interests include connective tissue biology, and regulation of bone and cartilage formation.

Dudley A. Saville, Ph.D. (Stephen C. Macaleer '63 Professor of Engineering and Applied Science, Chemical Engineering Department, Princeton University); research interests include fluid mechanics (electrohydrodynamics) and colloidal phenomena (electrokinetics and dielectric spectroscopy).

Howard A. Stone, Ph.D. (Gordon McKay Professor of Chemical Engineering and Applied Mechanics, Harvard University); research interests include transport phenomena and fluid mechanics (especially viscous flows and microfluidics) and flows involving complex fluids (e.g., foams).

Larry J. Suva, Ph.D. (Professor, Orthopedic Surgery Department, University of Arkansas for Medical Sciences); research interests include the relationship between bone blood flow and bone formation/resorption, and molecular mechanisms of bone cell function.

The panel was organized by Simon Ostrach, Ph.D., the Director of the National Center for Microgravity Research. Dr. Ostrach, whose research interests include fluid dynamics and heat and mass transfer in microgravity, also contributed to the panel's deliberations. The NCMR's Chief Fluids Scientist, J. Iwan D. Alexander, Ph.D. (Professor of Mechanical and Aerospace Engineering, Case Western Reserve University) also participated in the panel discussions and contributed to the report. Josée R. Adamson, M.Sc.E. (Associate Staff Scientist, NCMR) contributed as a liaison to integrate all communication aspects. The report issued by the panel was compiled and edited by Franklin T. Dodge, Ph.D. (Institute Engineer, Division of Mechanical and Fluids Engineering, Southwest Research Institute) whose research interests are in fluid dynamics and heat transfer in microgravity.

PANEL'S CHARGE AND PROCEDURES

The panel was asked to define critical areas of research in the biosciences where an understanding of the role of physicochemical processes such as flow and transport, force transduction, physical chemistry, and reaction kinetics is essential. In addition, the research areas were to focus on biological processes that are affected by the space environment (e.g., microgravity) and are crucial to NASA's mission.

The procedures followed by the panel were, in the main, the usual kind for a body that met only once during its existence. Each member of the panel was asked in advance of the meeting to prepare a list of promising research areas in which the physical and chemical sciences could enable advances in the biological and life sciences. As a result of the general discussion, it appeared that the promising research areas could be grouped into two general topics:

Cellular Physiology and Molecular Biophysics. Research areas include modeling, transport, mechano-biology, organs, plants, molecular mechanics, molecular sensors.

Medical and Biological Technologies. Research areas include devices, drug delivery, micro-analytic systems, and life support systems.

Following the meeting, the detailed work of the panel was coordinated electronically by e-mail, phone, or fax. The panelists prepared written summaries of the promising research areas in

detail. The summaries were integrated into this report, along with appropriate background material and recommendations. The report was distributed to the panel members, edited, and revised through several versions.

EXTERNAL REVIEWERS AND CONTRIBUTORS

A draft report was also circulated amongst five independent contributors whose expertise complemented that of the panel. In addition to reviewing the document, they contributed material to the report thereby extending and reinforcing the study of the panel.

Suneel S. Apte, MD, Biomedical Engineering Department, Cleveland Clinic Foundation

James P. Bagian, MD, NASA Astronaut (retired), Director, Veterans Health Administration's National Center for Patient Safety

James B. Bassingthwaighte, Ph.D. MD, Professor, Bioengineering Department, University of Washington

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Laura E. Niklason, Ph.D., MD, Assistant Professor, Biomedical Engineering Department, Duke University

Suggested Research Areas

BACKGROUND

To put the research areas recommended by the panel in context, the influence of physicochemical processes, microgravity, and the space environment on various biological phenomena is first reviewed briefly. Then, the approach of combined experimentation, modeling, and computation recommended for investigating these effects is discussed. Finally, the need for NASA's support of interdisciplinary educational programs in the physical, mathematical, and biological sciences is discussed.

Following the background material, each of the suggested research areas is discussed. The research areas are divided into two general topics, in accordance with the panel's deliberations. The list of suggested research areas is not meant to be exhaustive, and in most cases the research areas can be subdivided further; the intent is to indicate some of the important issues for NASA to consider.

Integrative Mechano-Biology in Microgravity

Much is known about systemic physiological responses in humans and animals to microgravity exposure, such as the shift of fluid from the lower to the upper body, the subsequent reduction in plasma volume and cardiovascular deconditioning, and the progressive loss of bone mass and alterations in calcium homeostasis[6,7]. Much less is known about the cellular and molecular mechanisms of these short and long-term responses or their genomic basis in man, animals and plants. Moreover, very little is known about how this fluid shift in microgravity influences drug delivery or action and other transport processes on the tissue scale or larger[8].

The major biological effects of microgravity are generally indirect and are typically mediated through alterations of a complex variety of physicochemical influences such as body forces, hemodynamic and hydrostatic pressures, fluid shear stresses, three-dimensional tissue stresses, and tissue transport and permeability[9,10]. Many of these physiological responses, such as altered levels of gene transcription, have cellular and molecular mechanisms that are becoming increasingly amenable to experimental probing. Nonetheless, the problem remains of how to integrate and understand this reductionist experimental detail with physiological function *in vivo*. To acquire the desired understanding of biological responses and adaptations to space environments, a multistep and multidisclipinary *mechano-biological* approach is required. This approach combines the modeling methods common in the physical and engineering sciences with the hypothesis-testing methods common in cellular and molecular biology, genomics and systems physiology. It should be emphasized however, that effective use of analytical and numerical models requires reliable physical property data for the particular system under investigation. Research programs that combine members from all these communities are an ideal way to bridge these two methods. Such a multistep, multidisciplinary approach, which involves modeling to guide experiments and experiments to revise and improve models, might take the following form:

- Formulation of idealized analytical models and scaling analyses in support of a specific biological hypothesis to identify the relevant levels of body forces and physicochemical effects (e.g., pH, ionic strength) needed to alter the system's response. This will require the application of systems engineering approaches including computational biology to predict how microgravity directly and indirectly influences the cells, tissues, and biological systems of interest. At the conclusion of this step, decisions could be made for further investigations.

- Formulation of more detailed models to support a finding that microgravity plays a role. These models would be augmented by a combination of experimentation and computation to establish the need for further investigations.

- Conduct of specific ground-based and space-based experiments and more complete modeling. These experiments and models would be aimed at assessing the ability of microgravity and the space environment to interact or interfere with the proposed processes and mechanisms.

The integrated, multistep, multidisciplinary approach recommended here offers new opportunities to answer basic scientific questions relevant to space and ground-based applications. Some examples of these questions are:

- How do cells sense and respond to mechanical forces?

- How do the direct versus the indirect physical effects of microgravity contribute to the biological responses and which effects dominate in different cell and tissue types?

- How are the distributions of physical forces in cells and tissues altered in microgravity?

- What are the cellular and molecular mechanisms of tissue-specific remodeling during prolonged microgravity exposure?

- How are cell differentiation, cell fate, and cell-cell and cell-matrix interactions regulated by forces and processes associated with gravity?

- What genes are activated or switched off by short and long-term microgravity exposure and what are the phenotypic consequences?

- Can specific allelic variants affect responses to microgravity?

- Can model organisms predict complex phenotypic consequences of prolonged microgravity or low gravity exposure?

- Can microgravity be used to help engineer tissue-specific responses *in vitro*?

Examples of areas in which analytical and computational modeling in addition to experimental research will be a critical part of the research include:

- Structurally-based models of cell and tissue biomechanics and transport processes including stress-dependent remodeling at the cell, tissue and organ scales.

- Biophysically-based mechano-biology models of mechanical, chemical, transport, and electrical aspects of cellular functions and interactions (e.g., mechano-electric feedback and mechano-chemical transduction).

- Experimentally data-driven and physicochemically-constrained systems models of cellular complexity, including network models of cell signaling; metabolic fluxes and responses to altered mechano-energetics; cell cycle; and gene networks.

- Theoretical and experimental models of force-regulated tissue growth, organ development, and morphogenesis.

- Integrative modeling of systems physiology including cardiovascular transport, whole body calcium homeostasis, and pharmacokinetics (e.g., integrative models of blood-tissue exchange of drugs, metabolites, substrates, and oxygen under normal and microgravity conditions).

- Evolutionary and developmental models for predicting pattern formation.

Education in Interdisciplinary Research

The past few years have seen a rapid growth of interdisciplinary programs at the undergraduate, graduate, and post-doctoral level that have the objective of integrating physical sciences, mathematics, and engineering with biomedical sciences. New graduate programs, mostly based in the engineering and natural science faculties, have been formed in bioengineering, biotechnology and bioinformatics. Existing programs have expanded their horizons beyond the traditional technology applications for medicine to include the application of engineering principles and methods to biological science. The establishment and growth of these interdisciplinary programs offers NASA a unique opportunity to include microgravity and space biology as an integral component of the programs and to educate the next generation of multidisciplinary scientists and engineers. The panel therefore recommends that NASA include interdisciplinary doctoral and post-doctoral training grants and fellowships as an integral component of its biological and life sciences research program.

RESEARCH TOPIC 1 – CELLULAR PHYSIOLOGY AND MOLECULAR BIOPHYSICS

Biological systems are subjected to physicochemical effects on nearly all aspects of their functions. These effects are evident at every level from the single molecule to proteins, enzymatic reactions, nano-scale forces in nanotubules, shear forces on lipid membrane, remodeling of bones, and fluid shifts within the body. A change from normal gravity to microgravity can therefore have a substantial effect on many physiological systems[9]. The effect may be direct (e.g., a change in body force or hydrostatic pressure) or it may be indirect through changes in physicochemical processes[11]. The consequences of these effects to interact or interfere with the processes by which cells divide, grow, function, remodel and respond to stimuli and injury need to be assessed by specific experiments, modeling, and computations. The panel identified several research areas in cellular physiology to aid in making these assessments, including cell response to the environment, cell differentiation, cell structure, and tissue remodeling. A major emphasis in all the suggested research areas is obtaining a better understanding of the physicochemical processes involved.

Cellular responses depend, at the fundamental level, on processes such as mechano-chemical transduction that occur on a *molecular* scale[12]. Understanding these underlying physical mechanisms remains a critical challenge, and progress in several affiliated research areas is a necessary step toward meeting it. Besides helping to understand the biological implications of long-term human space flight, molecular biophysics will also come into play for biosensor applications, instrumentation, and biologically derived materials.

Cell Response to the Environment

Microgravity causes significant changes in the distribution and flow of fluids within the body[6]. Consequently, cells experience changes in the fluid pressures that affect intracellular and extracellular shapes and volumes and alter the osmotic balance. Cytoskeleton changes occur directly through changes in cell shape and have important consequences for gene expression. The changes in fluid pressure can be sensed by *stretch receptors*, which have been found in the membrane of many types of cells in the body[13]. (Traditionally, stretch receptors refer to the stretch-activated K+ channels connected to the afferent fibers of the nervous system; it is likely that the cellular elements that sense mechanical stretch in other cell types do not use the same mechanism or at least can use other mechanisms.) Stretch receptors can produce a variety of responses to pressure changes, such as an increase in intracellular calcium concentration. Some cells are also capable of responding to changes in the osmolarity of the solution[14], and some ionic channels have been shown to be sensitive to osmolarity changes by altering their intrinsic properties. These may be merely the same channels, not activated by osmolarity but by stretch. Considering these observations, it is likely that stretch receptors are intimately involved in the effects of microgravity on cellular activity. However, stretch receptors have been poorly studied, especially in the nervous system. In particular, it is not clear which cells have stretch receptors nor what is the cellular response to a change in pressure. Since stretch receptors are probably located throughout the body, the distribution map of these receptors needs to be studied, and the various responses of the receptors to changes in fluid distribution and pressure induced by microgravity needs to be quantified.

Physicochemical processes involved. One of the primary physicochemical processes involved in the response of a cell to its gravitational environment is the pressure applied to cell membranes by the surrounding fluid and the stress consequently generated within the membrane[10].

Hypothetical involvement of microgravity. Microgravity directly affects the fluid pressure around a cell, which is sensed by stretch receptors embedded within the membrane of the cell, thus leading to modulations of cellular functions in microgravity and the space environment.

Model system for experiments. Several model systems are candidates for experiments, such as baroreceptors (found in the aortic arch and carotid sinuses) for which the stretch response is relatively well known, or smooth muscle cells, which are known to be stretch-activated. Another model system is dissociated cultured neurons of various types. This system can be investigated by the use of sealed chambers filled with the culture medium. To permit pressure changes within the physiological range, the chamber walls would need to be made of stretchable material, and the chambers would have to be capable of being rotated to cause fluid redistribution around the cell and pressure changes on the cell membranes. To monitor the response of the cells to pressure changes, the instrumentation should include an intracellular

calcium measurement system (fluorescent imaging or direct measurement of selected ionic currents) and a bioMEMS-patch clamp measurement system.

Cell Differentiation and Development

Nanoscale forces are applied to cellular cytoskeletal structures such as microfilaments and microtubules, through the cell's attachment to its pericellular and extracellular matrix. Perturbations of the cytoskeleton by microgravity can rearrange the molecular spacing or the accessibility of enzymes and their substrates, or kinases and their targets, permitting activation events to occur at the inner leaflet of the plasma membrane surface or on the surface of cytoskeletal structures[15]. The initial activation events would presumably cascade and amplify through conventional intracellular signaling mechanisms[16]. Physical attachment of the cytoskeleton to the nuclear envelope and nucleolus may also lead to the direct initiation of intra-nuclear activation events in the absence of second messenger cascades[17].

Physicochemical processes involved. The primary physicochemical process is hypothesized to be the nanoscale forces applied to cytoskeletal structures, which induce various activation events in the cell membrane.

Hypothetical involvement of microgravity. Microgravity is hypothesized to alter nanoscale forces and thereby to influence cell differentiation and development through the cell cytoskeleton.

Model system for *cell differentiation* experiments. Adult stem cells represent an ideal system to study the effects of the space environment on cell differentiation. These pluripotent cells are found in most adult tissues, they are crucial for local tissue repair and tissue maintenance, and they are mitotically quiescent and require an activation event to stimulate them to proliferate; when activated they will divide for 10 - 15 cell-cycles. Adult stem cells also exhibit asymmetric cell division, a process whereby pluripotent mother cells generate one pluripotent, quiescent daughter to replace itself, and another daughter cell that will proliferate as a progenitor cell for several generations; these progenitor cells then subsequently differentiate into phenotypically mature cell types such as bone, cartilage, muscle, liver, and adipose. Furthermore, stem cells can function either as single cells or in groups, thereby enabling an assessment of cell-cell interactions influencing differentiation.

Relevant outcomes for microgravity experiments are whether adult stem cells can be activated by specific stimuli (e.g., tissue damage), whether they can continue to exhibit asymmetric cell division thereby maintaining stem cell populations after tissue damage, and whether their progeny can differentiate into all possible mature cell phenotypes. The experiments should utilize a micro-systems approach since this will allow several BioMEMS micro-well (≤ 1 mm^2) stem cell cultures either as single cells or in small groups of stem cells, and the devices can be incubated in both orbit and ground-based environments in a minimum of volume. The stem cell cultures can be subjected to varying exogenous treatments to activate specific differentiation pathways, such as dexamethasone for bone or TGF-β1 for cartilage. Cultures can be assessed longitudinally for several metabolic properties via integrated sensing microelectrodes in the BioMEMS substrate material. Parameters to observe and measure could include time-dependent changes in native resistance, osmolarity, and pH gradients across the apical-basal axis of individual cells and groups of cells. In addition, glucose, oxygen, and specific ion flux measurements could be measured to test whether the cells exhibit changes in transport properties during differentiation. (These kinds of studies are limited by the existing

instrumentation and provide a compelling reason for investment in nanotechnology and microfluidics technology development.) At the end of incubation, the stem cell cultures can be scored microscopically for cell number and for the presence of differentiation-specific markers in each cell via immunostaining, *in situ* hybridization, or cDNA microarrays.

Model system for *cell development* experiments. *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster* represent ideal species to study the effects of space flight on developmental biology and to increase the understanding of adaptive responses in mature organisms. First, these systems are intact organisms that contain nearly all of the connective tissue phenotypes existing in higher order organisms. Second, both species exhibit rapid developmental cycles and produce several generations within a few months. Third, the developmental fate of each of the blastomeres within the early embryo of *C. elegans*, and the stem cells within the imaginal discs of *Drosophila* larvae have been accurately mapped, and are therefore fully accountable. Furthermore, the polytene chromosomes of *Drosophila* larvae salivary glands can be used to assay RNA transcription rates at the chromatin level by assessing the number and chromosomal positions of the RNA "puffs". Lastly, the technology exists to vitally label specific *C. elegans* blastomeres or *Drosophila* imaginal disc stem cells and follow their developmental fates under varying experimental conditions. Comparing the space environment to that of earth, relevant outcomes to investigate are determining (1) whether these developing organisms can generate similar numbers of progeny over several generations, (2) whether they exhibit normal temporal-spatial patterns of tissue development both in short- and long-duration space flight, and (3) whether alternative cellular and molecular mechanisms are employed to ameliorate potential developmental anomalies due to space flight.

For the same reasons given for the cell differentiation experiments, a micro-systems approach has advantages for cell development experiments. *C. elegans* embryo cultures can be subjected to real-time imaging with phase contrast microscopy, as all 959 somatic cells of its transparent body are visible. Though no bone tissue exists in *C. elegans*, 81 skeletal muscle cells generate flexing and bending movements in the adult organism. The development and function of these muscle cells can be directly measured by the video acquisition of movement activity of the 1 mm long adult organism. This aspect is relevant to the NASA mission as humans exhibit muscle atrophy and motor unit loss with increasing space flight duration. During a life cycle (roughly 2-3 weeks for *C. elegans*), the developing embryo cultures can be scored microscopically for cell number and for the presence of differentiation-specific markers in each cell via immunostaining, *in situ* hybridization, or cDNA microarrays after laser-capture of individual cells. It is realized that this may be difficult because of the amount of RNA available from single cells. *C. elegans* is ideally suited for cDNA microarray analysis since its diploid genome is encoded by 17,800 distinct genes which have been completely sequenced.

Plant Cell Function

Understanding the effects of microgravity on plant development may be crucial for long-term space flight success. Plants have various ways of sensing their mechanical environment, and the effects can be dramatic on plant phenotypic expression[18]. In some plants, relatively dense small particles will move in the cytoplasm in response to an altered gravity vector. The detailed signal transduction mechanisms involved at the cellular level caused by this movement and subsequent interaction with cell structures are still not completely understood. In addition, alterations of the plant loading can activate the expression of mechanically-sensing genes called TCH genes. The physiochemical mechanisms by which these mechanical force alterations are

transmitted to altered gene expression are not well known or understood, although mechanically responsive genes have been identified in *C elegans*[19] and in vertebrates[20].

Physicochemical processes involved. One of the primary physicochemical processes involved in plant cell functions is the motion of dense particles in the cytoplasm involved with the signal transduction at the cellular level and the expression of TCH genes[18]. Changes may be transduced to neighboring cells through ion fluxes[21].

Hypothetical involvement of microgravity. A change from normal gravity to microgravity has a direct effect on cell loading in plant structures. If this altered loading changes cell gene expression, altered plant phenotypes could result. In addition, sensory mechanisms dependent on density differences will be much less effective in microgravity.

Model system for experiments. The genetically best-characterized plant is *Arabidopsis*, so it is recommended for the initial model system. Studies in this system have already identified at least 5 TCH genes that are dramatically upregulated in expression by various environmental stresses, including mechanical forces. This mechanical stimulation can alter *Arabidopsis* developmental pathways. Since three of the five gene sequences identified as mechanically responsive in these plants appear to be calcium-binding proteins, calcium may play as important a role in mechanical sensing in plant cells as in animal cells. Another mechanically regulated gene encodes a cell wall modifying enzyme that is predicted to have roles in controlling cell growth.

Arabidopsis plants could be subjected to various types of specific force loading of individual plant structures. Detailed mathematical models incorporating the complex plant material structure and topology would be needed to estimate the local stresses at the individual cell level. Reporter gene constructs driven by TCH gene promoters would give load expression readouts that could be correlated with the calculated local mechanical stresses.

Cell Structure of Osteoblasts

The loss of bone mass in humans resulting from extended exposure to the space environment is an overarching concern for NASA and is a key area for future research[7]. It is not known at present whether exposure to the space environment causes diminished anabolic responses by bone-forming cells (osteoblasts)[22]. Furthermore, it is still unresolved as to how bone tissue actually forms; that is, how calcium and phosphate ions delivered via the vascular system achieve supersaturation concentrations for crystallization reactions in the extracellular matrix beneath osteoblasts (osteoid). It has been suggested that the osteoblast cell layer "seals-off" the underlying osteoid matrix from the overlying blood supply. This would suggest that the osteoblast cell layer forms a semi-permeable barrier between the vascular supply and bone tissue, thereby generating an extracellular matrix microenvironment within osteoid exhibiting chemical properties unique from matrix areas fully accessible to a vascular supply. Such a possibility could account for the unique efficiency and spatial regulation of calcification reactions in bone as compared to other connective tissues.

Physicochemical processes involved. Nanoscale forces are hypothesized to be applied to cytoskeletal structures such as microfilaments and microtubules through a cell's attachment to its pericellular and extracellular matrix.

Hypothetical involvement of microgravity. Microgravity is hypothesized to affect the structure of the plasma membrane junctional complexes, perhaps via changes in pressure difference across the cell junctions in a monolayer. Perturbations at the level of plasma membrane junctional complexes could rearrange the molecular spacing between adjacent cell surfaces and/or alter the molecular structures of these complexes thereby changing their permeability or transport functions.

Model system for experiments. Considering the remarks given above, osteoblasts are ideal cells to study the effects of space flight on cellular structure. Relevant outcomes to investigate in microgravity are (1) whether osteoblast cultures maintain their mature phenotype, (2) whether they continue to exhibit normal anabolic activities such as matrix synthesis and biomineralization of that matrix, and (3) whether osteoblast cultures can form and maintain functioning junctional complexes that would provide a semi-permeable barrier for the transport of fluids, ions, nutrients, and oxygen. In addition to the study of cultured cells on artificial substrates, complementary in vivo studies would be beneficial.

A micro-systems approach also provides advantages for this research area. Osteoblasts can be placed into a BioMEMS environment pre-coated with an adhesion substrate such as type I collagen, which is already pre-instrumented for assessing barrier functions. Such a device will allow these cells to acquire intimate cell-cell contact through high cell density growth. Several osteoblastic cell systems exist that achieve a tightly packed monolayer exhibiting cell-cell junctional complexes similar to that seen *in vivo*. Further, the BioMEMS environment will permit miniaturization (~1000 cells in 1 mm^2 wells), have integral pre-frabricated microsensors for multi-parametric, real-time measurements of cell polarity and barrier function, and permit the recovery of the cell layer for confirmatory morphometric and biochemical analyses. The miniature size will allow several BioMEMS micro-well osteoblast cultures to be incubated in orbit and in ground-based environments.

Observation or measurement made on parameters such as time-dependent changes in native resistance, osmolarity, and pH gradients across the apical-basal axis of the osteoblast monolayer should permit an assessment of a polarized cell layer. These outcomes will assess chemical polarization across the cell layer, and reveal the presence of oriented accumulation of molecules above or below the cell layer. Flux measurements on glucose, oxygen, calcium and phosphate flux measurements will be assessed in the absence of fluid flow to test whether the cell layer manifests semi-permeable barrier and ion-transport properties. In addition, cultures can be treated acutely with bone hormones (e.g., PTH or 1,25-dihydroxy-vitamin D3) to test for osteoblast responses with regard to polarity and barrier functions. Following real-time testing analysis, micro-well cultures can be recovered for assessing the quality and quantity of junctional complexes and bone matrix deposition via immunostaining, mineral analysis, Western blot analysis and gene chip analysis.

Tissue/Vascular Remodeling

Studies to date on physiological function under conditions of microgravity have highlighted the need for greater insights into the biophysical processes that govern transport at the tissue, cellular and molecular scales. Although many flight- and ground-based studies have been conducted[1], the regulation of fluid and solute transport remains poorly understood. A better delineation of factors that govern the convective transport of solutes within the circulatory and tissue compartments is needed, including the mechanisms that affect their normal regulation and adaptation to a microgravity environment. In addition, studies of tissue-fluid balance at the macrocirculatory level (large blood vessels that are greater than 1 mm in

diameter) and at the microcirculatory level (less than 1 mm in diameter and extending down to the size of a red blood cell) have not yet explained either the mechanisms for vascular volume shifts and compensatory vascular adjustments, or the effects of these adjustments on tissue transport to critical systems for the maintenance of blood volume and bone mass[23,24].

Physicochemical processes involved. Within the circulation, in both large and small blood vessels, convective transport of solutes is regulated in part by shear-dependent adjustments of vessel diameter. Blood imposed shear stresses may elicit cellular responses that govern not only the caliber of blood vessels, but the permeability barrier between blood and tissue compartment[24]. Diffusion processes are important determinants of the transport across the blood vessel wall and within the tissue space itself[25].

Hypothetical involvement of microgravity. The development of mechanical stresses in the walls of blood vessels due to postural changes, and the absence of such stresses in a microgravity environment, may influence the mechanisms that govern the shear stress dependency of vascular function. Previous studies have attempted to describe the potential for gravitational effects to influence myogenic and neurogenic mechanisms of vascular control and regulation, which depend on vascular distension and intravascular pressures. It is also possible that mechanical stresses along the length of large and small blood vessels, and their absence in microgravity, may influence known processes of mechano-transduction ascribed to constituent cells of the blood vessel wall. Stresses imposed on cells of the vascular wall by fluid transfer between vascular and tissue compartment may elicit vascular responses. Permeation of the tissue as a result of transvascular exchange of water and solutes may also be affected by gravitation effects and modification of the resistance to fluid flow within tissue in response to adaptation to microgravity. Gravitational effects on hydrostatic tissue pressure and ultrastructural rearrangements may affect vascular-tissue fluid balance and the subsequent transport of nutrients and metabolites in tissue. One of the main effects is the change in distribution of shear stress that accompanies the change in vascular diameter. That cardiovascular cell function is regulated by shear stress is well known, and the changes in vessel caliber (such as would occur in the veins of the lower extremities in microgravity) could be significant.

Model systems for experiments. Studies on vascular and tissue transport are needed that focus on events leading to long term tissue remodeling or acute compensatory alterations in circulatory function, the blood and tissue barrier, and transport within the tissue matrix. These studies need to emphasize the biophysical basis of cellular and molecular events and physicochemical transport. A model system for these studies is intravital microscopy of the living circulation, which is amenable to quantitative techniques for the measurement of convective fluxes in small blood vessels, permeability of solutes between blood vessels and tissue, and permeation of the tissue space by water and solutes. Ground-based models to date have employed exteriorized tissues of small mammals, which to some degree may mimic microgravity conditions, as in the suspended rat model. Although technical difficulties generally preclude flight experimentation on these mammalian systems, the potential for dual ground-based and flight experiments on the vascular system of the chick embryo appears to be feasible. Some studies have already explored circulatory transport dynamics and transvascular exchange in the chick chorioallantoic membrane (CAM); if these studies were fully exploited, they may shed new light on the role of physicochemical processes in transport and angiogenesis in a microgravity environment. Certain physicochemical processes may lend themselves to scrutiny using cell culture systems. In this case, processes such as regulation of transvascular exchange may be examined by studying permeability of cultured monolayers of endothelial cells. Flight experiments need to be designed to relate alterations before and after a specified

duration of microgravity, using technically feasible measurements on specially instrumented circulatory models; for example, the CAM can be instrumented to measure transvascular diffusion of solutes using specially designed photometric systems, and tissues can be instrumented with MEMS designed for electrochemical sampling and analysis. Computer simulations that integrate discrete observations at the molecular, cellular and tissue level are needed in concert with the experimental approaches.

Molecular Conformation, Order, and Structure

Changes in gravitational effects are manifested through changes in flow and pressure that result in changes in shear stress, strain, strain rate, transport, and direct hydrostatic pressure effects[12]. Mechanical stresses are known to alter cellular function through a signal transduction cascade leading to changes in gene expression, changes in synthesis and secretion of various factors, and a general stress adaptation response. The resulting changes in molecular order or structure are likely to play a role in mechano-chemical transduction. Reordering in the membrane may involve changes in packing density and phase of the lipid bilayer. Alternatively, forces can be transmitted to other locations in the cell such as the cytoskeleton or sites of focal adhesion where transduction may occur.

Physicochemical processes and hypothetical involvement of microgravity. Forces exerted on the cell can lead to localized changes in the conformation of the cytoskeleton and transmembrane proteins such as ion channels, integrins, and G-proteins. Fluid dynamic shear stress has been shown to produce changes in cell membrane fluidity and may consequently affect the rates of reaction between transmembrane proteins. In addition to mechanical forces, physical chemistry will play a role in influencing pH, ionic strength, charging of nearby surfaces, etc.

Computational methods. Numerical analysis will play a significant role in understanding the effects of microgravity on molecular structure. Computational methods for molecular dynamics are continually improving in accuracy and computational efficiency. The potential exists, or will soon be developed, to simulate the effects of altered environmental stress on individual proteins and to predict altered reaction rates. Even with improved computational algorithms, however, analysis of large protein structures will require new and novel computational approaches to reduce the number of degrees of freedom in the system or otherwise simplify the problem. Although experimental input will likely always be needed, the current computational methods need to evolve to a point where they are based more completely on fundamental biophysics. Development and judicious use of these methods is a critical step toward understanding the molecular bases for the spectrum of biological responses associated with a change in gravity.

Instrumentation and sensors. To characterize and measure mechano-chemical transduction processes quantitatively, new instruments and sensors and new applications of existing methods are needed to probe at the molecular scale. For example, existing methods such as fluorescence resonant energy transfer (FRET) and atomic force microscopy (AFM) could be extended to address these problems. Entirely new methods may well be necessary to visualize and measure phase transitions, protein activity, etc. New techniques for force application and molecular modification of surfaces are also needed. Other aspects of relevant molecular biophysics include novel methods for the design and/or fabrication of new materials (particularly those that are derived from biological sources or are protein-based), molecular self-

assembly, and the design of biosensors based on molecular recognition for biological monitoring in extra-terrestrial environments.

Genomic Research

New technologies in high-throughput and functional genomics have created opportunities for multidisciplinary research that can elucidate the basic biological mechanisms underlying *in-vivo* responses to microgravity. With these new technologies, researchers can investigate and identify the genes – or more specifically, the networks of genes and their products – whose expression and function are regulated and affected by physical forces and the other changes associated with space environments[25]. Techniques such as gene expression profiling with large-scale microarrays combined with careful statistical and clustering analysis can be used to identify genetic circuits that are modulated by physical forces *in vivo* or *in vitro*[26]. As an example, such research might answer the question as to what genetic pathways are altered during cardiovascular and musculoskeletal deconditioning.

Physicochemical processes involved. Ground based research has demonstrated that gene expression and cell phenotype, including cell differentiation and cell fate, are sensitive to the mechanical environment. Genetic circuits are affected by physical forces *in vivo* and *in vitro*.

Hypothetical involvement of microgravity. The changes in the mechanical environment (e.g., fluid pressure) of the genes in microgravity can modulate their expression.

Model system for experiments. Gene targeting of biomechanical signaling pathways that have been identified by controlled *in-vitro* studies should be investigated *in vivo*. For example, studies of tissue-restricted or conditional gene "knock-out" studies in mice, which have illuminated the molecular basis of complex *in-vivo* conditions such as atherosclerosis and heart failure, could be used to assess the role of key genes in acute and chronic microgravity responses *in vivo*[27]. Novel high-throughput functional assays of model organisms with tractable genomes such as *D. melanogaster* and *C. elegans* can be used to screen for allelic variants that might be involved in adverse responses, and thus identify therapeutic targets. These functional screens promise to provide particularly valuable insight into otherwise intractable problems such as the possible effects of long-term microgravity exposure on aging or development. Some promising experimental approaches to these problems include:

- Novel methods of instrumentation and fabrication aimed at probing the cellular and molecular responses of cells to physical stimuli, including protein sensors and techniques for applying forces at molecular scale (e.g., single molecule mechanics for elucidating the structural basis of mechano-chemical signaling).
- Microfabrication and microfluidics for miniaturized analytical systems, biomedical devices, and microscale cell engineering.
- Physical systems for controlling the mechanical environment of isolated cells and determining mechanical properties and stress distributions.
- New techniques for engineering *in-vitro* cell culture models of microgravity (e.g., soft lithography for traction force microscopy and cell sorting).
- Automated high throughput physiological phenotyping in engineered cell systems and model organisms.

RESEARCH TOPIC 2: MEDICAL AND BIOLOGICAL TECHNOLOGY

Drug delivery and action depend on transport processes such as flow, diffusion, and dispersion and are further impacted by electrostatic effects (pH, ionic strength), both at the relatively large (macroscopic) scale of capillaries and tissues and the relatively small microscopic scale of cells and molecules. A microgravity environment can therefore have a significant effect on drug delivery and drug action[8], if for no other reason that microgravity has a significant influence on the distribution of fluids in the human body and related changes in transport pathways. A specific example of this effect on drug delivery is inhaled aerosols, which may be affected directly by the change in gravitational sedimentation.

Novel devices are needed both for cellular-level experiments and for the controlled study of drug delivery and function. It appears that *microfluidics* may be such a crucial enabling technology. However, the small size of microfluidics devices means that their function can be critically affected by physicochemical phenomena (e.g., surface charge); hence, although microfluidics devices offer unique possibilities for sensing and control, their design and function must account for the physicochemical environment in which they will be expected to operate. Furthermore, while the principal goal is to further the health of the astronauts, an added opportunity exists: to exploit novel monitoring systems in order to collect data in simpler living organisms and to augment experimental efforts in modeling and cellular physiology.

Finally, the overall function of the working environment in space should be studied as this affects the quality of the air and water. A coordinated database must be established for ready access by the scientific and engineering communities.

Drug Delivery and Bioavailability

In a space environment, such common activities as chewing, swallowing, esophageal reflux, gastro-intestinal functions, and drug clearance are all altered[28]. Thus, unique issues arise in terms of drug delivery, drug safety, and the formulation of orally active compounds. Microgravity will influence many of the changes in metabolism and cardiovascular physiology which may significantly alter drug delivery. Efforts to study and quantify these changes are important for health care in space. Space-based and ground-based research on the improved rational design of active formulations for optimal performance is needed to support long-term human space exploration.

Effects of microgravity. The environment of microgravity and long-term space flight influences the medical management of astronauts[29]. In part, this is a result of the well-documented shift of fluids in the human body when exposed to microgravity.

Physicochemical processes involved. Drug delivery depends on fluid dynamics and chemical diffusion. For example, transdermal technologies involving electroporation or sonoporation are sensitive to the changes in interstitial fluid content in dermal layers caused by microgravity. Likewise, inhalation technologies, which depend on particle transport and deposition in lungs, are altered relative to the terrestrial environment under conditions of microgravity and may impact their physiologic effect.

Research areas and suggested experiments. Drug delivery in a space environment is an area in which engineering and transport modeling will be critical. For example, dry-air-driven powder injection may have utility during medical treatment of astronauts, but like many delivery methods, its pharmacokinetics in space is unknown. These novel or redesigned delivery systems must exploit the growing knowledge of drug biodistribution and drug activity in humans experiencing microgravity and the space travel environment.

Pharmacokinetics and Pharmacodynamics

Both the pharmacokinetics (what the body does to the drug) and the pharmocodynamics (what the drug does to the body) of drugs in space should be studied. Pharmacokinetics is the study of the time course of drug and metabolite levels in the fluids, tissues, and excreta of the body, and the mathematical relationships required to develop models to interpret such data. Pharmacokinetics studies in space provide unique opportunities for improving astronaut health care and advancing our understanding of pharmacokinetics on earth. Even the effectiveness of common painkillers and their optimal doses in microgravity compared with Earth are in need of research. In terms of improved medicine for astronauts, it is critical to apply stochastic control theory to the design of optimal dosage regimens in microgravity. Modeling of these processes is desirable, and must be combined with experimental measures and outcomes: decades of work has shown that subject-to-subject variability can confound even the best modeling efforts. This will require improved methods for pharmacokinetics modeling and the investigation of process and measurement noise in the space environment. The research therefore will require by necessity interaction among biologists, engineers and system modelers.

Effects of microgravity. Just as for the delivery of drugs, pharmacokinetics is affected by changes in fluid transport, the local chemical environment, and human responses to the microgravity environment.

Physicochemical processes involved. Drugs are transported in fluids, tissues, etc., by a variety of fluid flow and diffusion processes, and some drug delivery methods utilize novel physicochemical processes such as localized electric or sound fields.

Research areas and suggested experiments. The impact of space on pharmacokinetics will require the evaluation of alternative pharmacokinetics models and optimal monitoring strategies for pharmacokinetics studies in animals and humans. An important outcome of these investigations would be the simultaneous development of clinically reliable interfaces and apparatus for delivering and monitoring therapeutic agents in space. Efforts in this area will allow the support of long-term space flight and provide the basis for improved astronaut care.

Medical Devices and Technology

The development of in-flight technologies and devices that are compact and non- or minimally-invasive and that can be used to assess parameters of pharmacokinetics following drug delivery orally, transdermally, or by inhalation would serve a valuable role in enhancing modalities for healthcare of astronauts. A number of factors are needed to drive the development of new medical device technologies for space. Of paramount importance is the necessity for a better understanding of the functioning of the human body in space and the influence exerted by microgravity. Medical devices for space should take advantage of stronger, lighter materials, nanoscale components, and faster processing capabilities. A significant challenge in designing these devices is to develop processing techniques for natural biomaterials or to develop materials that combine the advantages of synthetic materials with the activity of natural biomaterials. The development of these devices will encourage the effective application of therapeutic drug monitoring in space, with the aim of optimizing the clinical utility of therapies from Earth and maximizing astronaut health benefits.

Effect of microgravity. Depending on the device, gravitational effects may not be of concern. For example, because of their small size, microfluidics devices are not influenced in

any substantial way by gravitational forces but are impacted substantially by common physicochemical forces (e.g., surface charging).

Physicochemical processes involved. At the level of a device, the physicochemical processes involved are flow, diffusion, and pressure. At times, surface forces and intermolecular forces may also affect the operation of the device.

Research areas and suggested experiments. Much of the emphasis in current medical research is not on finding cures for disease, but rather on developing new methods for predicting and preventing disease. Research in this area is critical for astronaut safety. For example, the application of microarray analysis to humans and animals in space will increase our understanding of the impact of microgravity on biological systems. In addition, advances in tissue engineering are required and can be expected to play a significant role in new therapies in the coming decades. In essence, tissue engineering involves the application of the principles of biology and engineering to the development of substitutes capable of restoring, maintaining, or improving tissue function.

Sensors will play a pivotal role in transforming astronaut health care. Conventional earth-designed laboratory testing could be completely replaced in space by astronaut sensors (either worn or implanted). The space environment might be equipped with sensors to initiate automatic responses to astronaut medical needs. Sensor technology research and application is a critical component of the interactions among physical sciences, engineering and biology. Combined with artificial neural networks, they may form the basis for developing "smart" devices capable of providing functional support to astronauts and clinicians. The technology used to produce and test micro-biosensors is an area requiring significant research effort and is part of a larger development effort in microfluidics technology.

Microfluidics

The understanding, design, and fabrication of micron-scale flow devices (i.e., microfluidics) offer many opportunities for enhancing biological and medical science and technology[30]. Microfluidics devices can be designed using silicon micro-machining methods. Soft lithography techniques can also be used to effect very rapid designs with the added feature that the device itself is flexible, since it is made from deformable elastomeric materials[31]. These two fabrication techniques can be used to make a wide variety of mechanical components for manipulating fluids, cells, and biopolymers such as DNA and can be integrated with sensors and other devices.

Microdevices allow manipulation with fast response times, and can handle small fluid volumes, sense and control flows, and pattern substrates on small length scales; moreover they can selectively address the cellular scale. A variety of valves, pumps, actuators, and mixers has been developed. Fluids are driven through the devices by pressure gradients or electric fields. The integration of these elements is the basis for the development of micro-total-analytic systems, which are aimed at the development of a “laboratory on a chip”. Many of these ideas are now being applied in biological laboratories and biomedical companies (DNA chips, protein chips, high-throughput screening, etc.). There are thus a wide variety of possibilities for using these components, designing new components, and utilizing this kind of technology for furthering NASA’s research objectives.

Effect of microgravity. Because of the reduced scale of microfluidics devices, they are not influenced by gravity in any substantial way.

Physicochemical processes involved. Microfluidics devices depend on fluid transport and the methods used to drive the transport. Surface forces, intermolecular forces, and interfacial tension may also affect their operation.

Research areas. Because microfluidics devices are designed on the micron (and smaller) scale, they can be used for experiments involving drug delivery or as measurement systems on the scale of the cell. For example, microchannels can be used to deliver drugs selectively to individual cells, or even different parts of a single cell. Conversely, the channels can sample the fluid environment in the neighborhood of individual cells. Since an individual cell appears to be sensitive to its mechanical environment (e.g., the substrate to which it is attached and the local stress acting on the cell membrane), the mechanical environment of the cell must be considered carefully when drawing conclusions about the response of cells studied with these systems. Imaging techniques can be integrated to provide direct optical access to individual cells or colonies of cells. Fluids can be directed through complex networks of channels allowing small quantities to be analyzed in real time. All these features impact the science and technological objectives of many biological and medical experiments. Thus, microfluidics science and technology needs to be encouraged and integrated into research (e.g., understanding, manipulating and sensing cellular and tissue level processes). Moreover, microfluidics devices can be an integral part of "smart" and "active" systems for monitoring and controlling the contained environmental health and safety of NASA equipment and stations.

Contained Environmental Health and Safety

Research on recycling, self-generating, self-cleaning, and self-monitoring systems is needed to maintain more effectively a healthy working environment for astronauts. Research in "smart" and "active" systems for monitoring the environment is needed to detect and eliminate potentially unhealthy environments.

Effects of microgravity. Some familiar earth-based filtering and sensing technologies of small particles depend on gravitational settling, and are hence ineffective in microgravity.

Physicochemical processes involved. The processes involved in monitoring and cleaning the environment of an inhabited spacecraft include fluid transport, diffusion, settling, and filtering.

Research and development areas. Research and development is needed at the system level to better integrate health-related sensing elements into water and air filters (e.g., to detect hazards), and to develop procedures for self-cleaning filters, valves, air and water delivery devices, and other on-board features. Microfluidics devices, because of their small size and capacity for integration into laboratory-on-a-chip modules, would be potentially very useful for helping to monitor the working environment. There are potential opportunities to design and use functionalized surfaces (e.g., anti-bacterial surfaces; pressure and heat-sensitive). "Intuititive" sensors that offer small size, and increased stability, reliability and accuracy are needed to complement a physician's efforts on Earth to provide astronauts with immediate healthcare information.

Bioinformatics

NASA's program in biological and life-sciences research can be enhanced by the availability of pertinent results from previous and on-going research. Therefore, electronic database design and the data management of experimental results should be given high priority in the preparation of biological research projects and NASA's project review and selection. The databases should be linked to existing comprehensive databases of gene, proteins, structures, and expression. Development of these databases should consider the following observations.

The major experimental format for genomic study involves DNA microarrays, DNA membranes, and light-addressed oligo synthesis on chips. The major experimental format for proteomic study involves two-dimensional gel electrophoresis/mass spectroscopy, as well as peptide-chips and antibody-chips which will require further validation. Studies of whole-organism endocrine functions rely on a suite of individual clinical assay techniques that have yet to be unified at a biochip level. Statistical interpretation of data sets generated from these techniques is an ongoing effort. While the response to microgravity may not be limited to a single "microgravity phenotype" or "microgravity gene response element", improved understanding of humans in microgravity remains a critical goal and will require knowledge of hundreds to thousands of distinct gene regulation events that may occur in various organs. A typical phenotypic study involving four groups of 10 mice (control and transgenic strain on earth and in microgravity) can generate 60 million data points or images [e.g., 40 mice X 10 organs X 50,000 cDNA microarray X (2 colors + 1 background)]. Along with biological microsamples obtained daily from humans during space travel, a single mission with 10 to 20 biological experiments could generate 10 to 100 billion pieces of bioinformation specific to the microgravity environment.

A NASA-maintained database web site will be an invaluable international resource. It would be a permanent archive of all peer-reviewed scientific papers and linked databases generated from space missions, declassified project reports, and medical reports. Obviously, special attention to confidentiality issues would be required for data derived from medical tests on humans.

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Appendix A. Effects of a Space Laboratory

The space environment has an influence on the design and interpretation of biological and biomedical experiments in microgravity. Several effects that should be considered, including:

- **Non-uniformity of gravity field**. Although many of the suggested investigations require only that gravity forces be small, some experiments may be sensitive to the actual level of the gravity forces. The effective gravitational field on the International Space Station is of the order of at least $10^{-5}g_0$, where g_0 is the standard earth gravitational constant. The effective gravitational field can also vary by about $10^{-6}g_0$ over a distance of one meter; this variation also occurs on earth where it is negligible, but in space the variation may be of the same order of magnitude as the overall effective gravity level.

- **G-Jitter**. The effective gravitational field varies with time in deterministic and random ways, as a result of mechanical vibrations, astronaut motion, and other routine occurrences. The level of the vibrations can be as large as $10^{-3}g_0$. The disturbances impose vibrating forces on the experimental apparatus, and if the apparatus contains fluids, the fluids may slosh thereby inducing other disturbances to the experiment unless means are used to control the sloshing.

- **Cosmic radiation**. High-energy cosmic particles impinge on the International Space Station continuously. If an experiment is susceptible to radiation effects, it must be shielded.

- **Noise and atmospheric quality**. Experiments that involve animal models need to consider whether the overall laboratory environment has an influence on the responses of interest.

- **Instrumentation**. Biological and biomedical experiments in space will not generally have access to all the analytical instruments commonly found in a terrestrial laboratory. If not, methods must be used to transmit any needed data in near real-time for analysis on earth.

- **In-space Investigator**. The crewmember conducting the in-space experiment will, in many cases, not be an expert in the field of the investigation, although he/she will be trained in the conduct of the experiment. Consequently, subtleties that would be noticed by the Principal Investigators who devised the experiment may go unnoticed by the crewmember. For that reason, methods should be used to ensure that complete data histories are obtained for later examination, and the Principal Investigators should be in contact with the crewmembers periodically to discuss the progress of the experiment.

Appendix B. Glossary

Anabolic Relating to, characterized by, or promoting anabolism (i.e., constructive metabolism).

Angiogenesis The process of vascularisation of a tissue involving the development of new capillary blood vessels.

Arabidopsis A genus of flowering plants found in north temperate regions. The species *a. Thaliana* is used for experiments in classical plant genetics as well as molecular genetic studies in plant physiology, biochemistry, and development.

Bioinformatics The study of the flow of information in living systems, and the analysis of biological data.

BioMEMS The use or development of microelectromechanical systems for biological applications.

Blastomeres The undifferentiated cells formed by cleavage of the fertilized ovum.

Caenorhabditis elegans Nematode used in lineage studies since the number of nuclei is determined and the nervous system is relatively simple.

cDNA Complementary DNA: DNA that is synthesized from a messenger RNA template. The single-stranded form is often used as a probe in physical mapping to locate the gene or can be cloned in the double stranded form.

Chorioallantoic membrane Extra-embryonic membrane formed in birds and reptiles by the apposition of the allantois to the inner face of the chorion. The chorioallantoic membrane is highly vascularised and is used experimentally as a site upon which to place pieces of tissue to test their invasive capacity.

Cytoplasm: All of the substance of a cell outside of the nucleus.

Cytoskeleton That part of the cytoplasm that remains when organelles and internal membrane systems are removed.

Differentiation (cell) Progressive restriction of the developmental potential and increasing specialization of function which takes place during the development of the embryo and leads to the formation of specialized cells, tissues, and organs.

Drosophila melanogaster A species of fruit fly much used in genetics because of the large size of its chromosomes.

Electroporation Method for temporarily permeabilizing cell membranes so as to facilitate the entry of large or hydrophilic molecules as in transfection. A brief (about 1msec) electric pulse is given with potential gradients of about 700V/cm.

Endocrine Pertaining to internal secretions, hormonal.

Endothelium: A layer of flat cells lining the closed internal spaces of the body such as the inside of blood vessels and lymphatic vessels.

G protein GTP-binding proteins, often located in the cell membrane and implicated in the transduction of mechanical force into a biochemical signal by a cell.

Genome The total set of genes carried by an individual or cell.

G-jitter The unwanted (usually) vibrations felt in space shuttle and space station experiments caused by a variety of mechanisms, including astronaut motion and exercise, machinery, and engine firings; g-jitter has both random and deterministic components.

GTP-binding protein Regulatory proteins found in cells; they are versatile molecular switches involved in the control of a wide range of biological processes; they all act through a common molecular mechanism based on their ability to bind the guanine nucleotides gtp and gdp selectively.

Homeostasis A tendency to stability in the normal body states (internal environment) of the organism. It is achieved by a system of control mechanisms activated by negative feedback; for example a high level of carbon dioxide in extracellular fluid triggers increased pulmonary ventilation, which in turn causes a decrease in carbon dioxide concentration.

Imaginal disc Epithelial infoldings in the larvae of holometabolous insects (e.g., Lepidoptera, Diptera) that rapidly develop into adult appendages (legs, antennae, wings, etc.) during metamorphosis from larval to adult form.

Immunostaining The use of antibodies conjugated with fluorescent or histochemical dyes to label specific protein molecules for microscopy.

Integrins Superfamily of cell surface proteins that are involved in some cases in binding to extracellular matrix components that are involved in cell adhesion.

Intravital Relating to actions performed upon or occurring while a subject is alive.

Ion channel A transmembrane pore that presents a hydrophilic channel for ions to cross a lipid bilayer down their electrochemical gradients. Some degree of ion specificity is usually observed and typically a million ions per second may flow. Channels may be permanently open like the potassium leak channel, or they may be voltage gated like the sodium channel, or ligand gated like the acetylcholine receptor.

Kinase Abbreviation for phosphokinase, an enzyme catalyzing transfer of phosphate from ATP to a second substrate, for example creatine kinase.

Knock-out (gene) Organism where one or more genes have been specifically removed from the genome; in mammalian systems, a mouse is usually used.

Mechano-Biology The interface between mechanics and biology, generally used in the context of a biological response elicited by a mechanical stimulus; also, the study of biological responses to mechanical influences.

Mechano-chemical transduction The processes by which cells convert mechanical stimuli or forces to biochemical signals (also called mechano-transduction)

Mechanoenergetics The processes involved in converting metabolic energy to mechanical work or motion in cells and tissues, organs and organisms, e.g., muscle

Metabolic Of or pertaining to metabolism.

Metabolism The sum of all the physical and chemical processes by which living organized substance is produced and maintained (anabolism) and also the transformation by which energy is made available for the uses of the organism (catabolism).

Metabolite Any substance produced by metabolism or by a metabolic process.

Microfluidics Refers to fluid motion at the micron and small length scales, which is generally the realm of low Reynolds number hydrodynamics where viscous effects in the flow are important.

Microgravity phenotype (gene response) The characteristics displayed by an organism in a microgravity environment, regardless of the actual genotype of the organism.

Morphogenesis The process of shape formation that produces the complex shape of an adult from the simple ball of cells derived from the division of the fertilized egg.

Myogenic Pertaining to a family of muscle-specific transcription factors that bind DNA in control regions and thus regulate myogenesis.

Nucleolus A small dense body (sub organelle) within the nucleus of eukaryotic cells, visible by phase contrast and interference microscopy in live cells throughout interphase. Contains RNA and protein and is the site of synthesis of ribosomal RNA. The nucleolus surrounds a region of one or more chromosomes (the nucleolar organiser) in which are repeated copies of the DNA coding for ribosomal RNA.

Neurogenic Pertaining to or arising from or caused by the nervous system.

Oligo-synthesis The synthesis of DNA by addition of individual bases (monomer units); single stranded DNA molecules synthesized in this way are typically called "oligos."

Osteoblasts Cells that arise from fibroblasts and which, as they mature, are associated with the production of bone.

Osteoid Uncalcified bone matrix, the product of osteoblasts; consists mainly of collagen, but has osteonectin present.

Patch clamp measurement system The use of a micropipette, attached to the cell membrane by means of negative pressure, to isolate and make measurements of transport of ions or other molecular species across the membrane.

Pharmacokinetics The action of drugs in the body over a period of time, including the processes of absorption, distribution, localization in tissues, biotransformation, and excretion.

Phenotype The total characteristics displayed by an organism under a particular set of environmental factors, regardless of the actual genotype of the organism; results from interaction between the genotype and the environment.

Physicochemical Processes that involve both physical and chemical phenomena.

PTH Parathyroid hormone, which is important in bone homeostasis.

RNA A nucleic acid found in all living cells; it plays a role in transferring information from DNA to the protein-froming system of a cell.

Receptor A molecular structure within a cell or on its surface; characterized by selective binding to a more mobile substance (e.g., ligand) and a specific physiologic effect that accompanies the binding.

Somatic Pertaining to or characteristic of the soma (body) or body wall in contrast to the viscera.

Sonoporation The use of low-frequency ultrasound to enhance transdermal delivery of drugs; the ultrasound increases the permeability of the skin and causes the transport rates to increase.

Stretch receptor A receptor that is activated as a consequence of local deformations (strains) in the tissue.

TCH genes Touch sensitive genes.

TGF-β1 The transforming growth factor beta, which is a protein secreted by many different cell types; it stimulates wound healing but *in vitro* is also a growth inhibitor for certain cell types; in general TGF proteins are secreted by transformed cells that can stimulate growth of normal cells.

Transcription Synthesis of RNA by RNA polymerases using a DNA template.

Transdermal Entering through the dermis or skin, as in administration of a drug applied to the skin in ointment or patch form.

Transduction See *mechano-chemical transduction*

Transmembrane protein Proteins that span the cell membrane, having both intracellular and extracellular domains; the polypeptide chain is exposed on both sides of the membrane.

Western blot A technique used for searching for specific proteins.