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Versatile Fluid-Mixing Device for Cell and Tissue Microgravity Research Applications

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Microgravity life-science research requires hardware that can be easily adapted to a variety of experimental designs and working environments. The Biomodule is a patented, computer-controlled fluid-mixing device that can accommodate these diverse requirements. A typical shuttle payload contains eight Biomodules with a total of 64 samples, a sealed containment vessel, and a NASA refrigeration–incubation module. Each Biomodule contains eight gas-permeable Silastic T tubes that are partitioned into three fluid-filled compartments. The fluids can be mixed at any user-specified time. Multiple investigators and complex experimental designs can be easily accommodated with the hardware. During flight, the Biomodules are sealed in a vessel that provides two levels of containment (liquids and gas) and a stable, investigator-controlled experimental environment that includes regulated temperature, internal pressure, humidity, and gas composition. A cell microencapsulation methodology has also been developed to streamline launch-site sample manipulation and accelerate postflight analysis through the use of fluorescent-activated cell sorting. The Biomodule flight hardware and analytical cell encapsulation methodology are ideally suited for temporal, qualitative, or quantitative life-science investigations.

Introduction

DURING the last two decades, a number of adaptive physiological and biochemical responses have been observed when living organisms are exposed to a reduced gravitational field.[1–8] In humans, these changes include alterations in vestibular function, bone demineralization, muscle atrophy, cardiovascular deconditioning, suppressed immune function, and dermatological disorders, as well as alterations in fluid balance and hormone secretion. These observations demonstrate that space may provide a unique and informative experimental model for evaluating complex regulatory processes.

Although the scientific community is expressing increased interest in microgravity experimentation, the high cost associated with this research and NASA's austere research budgets present formidable problems for an expanded microgravity research program. To sustain the interest of the scientific community, mechanisms must be found to accommodate larger numbers of investigators at a time when flight opportunities continue to decline. In addition, individual investigators must have the flexibility to develop experimental designs with variable timelines and then perform these carefully controlled experiments with adequate numbers of samples for statistical reliability.

Over a period of thirty years, a variety of different cell culture systems have been developed for microgravity investigations.[8–11] Unfortunately, most of the cell biology flight hardware has been developed by the European Space Agency for vehicles other than the Shuttle.[8,9] Recent hardware efforts have focused on the development of small middeck payloads that provide greater flexibility and more frequent flight opportunity.[10,11] In this report, we describe newly developed flight hardware that provides excellent environmental control and exceptional versatility for life-science experimentation. In addition, improved analytical methods are introduced for the postflight analysis of limited quantities of biological samples. The hardware described in this report can simultaneously accommodate the unique experimental requirements of several investigators, thereby providing greater science yield at a lower cost.

Biomodule Operation and Design Features

A list of the Biomodule design features is presented in Table 1. Historically, the concept for the Biomodule began with the objective of being able to automatically transfer a test solution from a storage container to a sample. This basic design objective eventually led to the development of the prototype depicted in Fig. 1. The Biomodule was initially flight tested in 1989, on Consort 2, and subsequently patented.[12] To date, the Biomodule has flown on five sounding rockets[13] and on one mission aboard the Space Shuttle.

Each Biomodule accommodates eight samples. It weighs ≈0.54 kg, occupies ≈844 cm^3 of space (external dimensions $h \times w \times l$, $= 7.5 \times 7.5 \times 15.0$ cm), and it utilizes 2.5 A of current for a period of 300 ms during operation (i.e., during fluid transfer). Routinely, five to eight Biomodules or 40–64 samples are flown per mission. The compact modular design enables the hardware to be easily adapted to a variety of different launch vehicles. In addition, the Biomodule payload is autonomous in its operation, and it needs no hands-on attention from the astronauts following activation.

The bottom panel of Fig. 1 illustrates how the test solutions and samples are configured within a T tube. The biological samples are housed within a T-shaped container manufactured from a medical-grade Silastic elastomer. The positioning of the yoke bars to their closed configuration (lower panel of Fig. 1) creates three separate compartments within the T tube into which test solutions or biological sample can be added. In most applications, the samples are added to the sample chamber located in the stem of the T tube while the test solutions are added to the two lateral compartments created by the positioning of the yoke bar to its closed configuration. On orbit, a computer is employed to activate a solenoid to move the yoke bar from its closed to its open configuration. The unlatching of

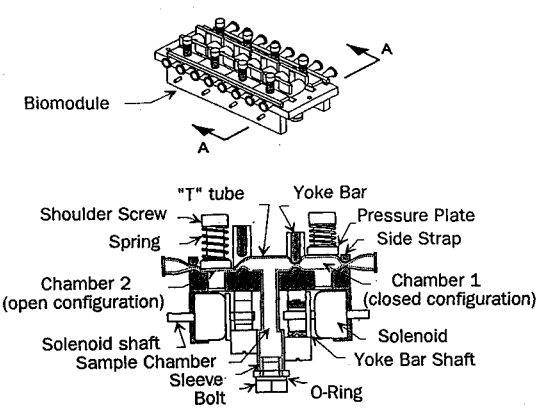

Fig. 1 Cross-sectional view of the Biomodules, section A-A.

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the yoke bar initiates the transfer of the pressurized fluids contained within the side chambers to the sample chamber. A step-by-step summary of the loading and operation of the Biomodule is given in Fig. 2.

Prior to the shipment of the hardware to the launch site, eight sterile disposable T tubes are inserted into each Biomodule and appropriate adjustments are made to the yoke-bar latching mechanism to achieve optimal hardware performance. The Biomodules are then wrapped and gas sterilized.

At the launch site, the Biomodule solenoids are test fired within their sterile wraps to confirm that the latching mechanism has not been disturbed during shipment. After a successful test firing, each Biomodule is unwrapped within the confines of a sterile laminar-flow hood, and the yoke bars are carefully latched in their closed configuration (see Fig. 1). The Biomodule is then placed on its side, and 0.15 ml of an appropriate test solution is added to chamber 1 through the open end of the T tube. This process is repeated until all eight T tubes are loaded with test solution. At this point, the side strap is closed, thereby creating a distinct fluid-filled compartment within each of the eight T tubes, as depicted in Fig. 2a. The Biomodule is then repositioned and the previous steps are repeated in order to fill the contralateral chambers with test solutions (Fig. 2b). Depending on the stability of the test solutions, these fluids can be loaded into the T tubes several days prior to launch.

Table 1 Biomodule design features

Eight sample chambers per Biomodule.
Sample chamber (0.7 ml); side chambers (0.15 ml). Larger sample volumes are possible.
User-defined, computer-controlled addition of two test solutions to the sample chambers.
Active mixing of two test solutions within the sample chamber.
No cross contamination; all samples are housed within individual T tubes.
Silicone T tubes are gas permeable, thereby providing gas diffusion into and away from the samples.
T tubes are autoclavable and nontoxic to biological samples.
Biological samples include bacteria, cells (suspension or substrate-dependent cultures) or tissue (plant or animal).
Multiple investigators can simultaneously load individual Biomodules, thereby eliminating confusion and reducing sample processing time at the launch site.
Hardware is adaptable to any launch vehicle.

Shortly before the hardware is turned over for integration into the launch vehicle, the biological materials are added to the sample chambers. The Biomodule is positioned so that the opening of the sample chamber is in an upright position, and 0.7 ml of sterile medium is added to each sample chamber. Cell suspensions can be added along with the medium. In cases where substrate-dependent cell cultures are employed, coverslips, with their attached cells, are added to the sample chambers after medium addition. The sample chambers can accommodate bacteria, cell suspensions, or tissue samples. After the samples are loaded, a sterile plug is screwed into the plastic sleeve to confine the fluids to the sample chamber. A coverslip with its attached cells is depicted in the medium-filled T-tube compartment in Fig. 2c.

After all of the sample chambers are securely sealed, the external openings of the T tube are cleaned with a disinfectant and the Biomodule is transferred to an engineer for installation into the flight hardware (containment vessel). The pressure-plate assembly is attached to the Biomodule (Fig. 2d), and a final check of the yoke bar is performed to confirm that the latch mechanism is securely seated. The Biomodules are then bolted into the hardware and connected to the onboard computer that controls the timing of all on-orbit fluid transfers.

In addition to initiating the fluid transfer functions, the onboard computer also collects temperature data and performs other housekeeping activities. Current computer designs provide for significant

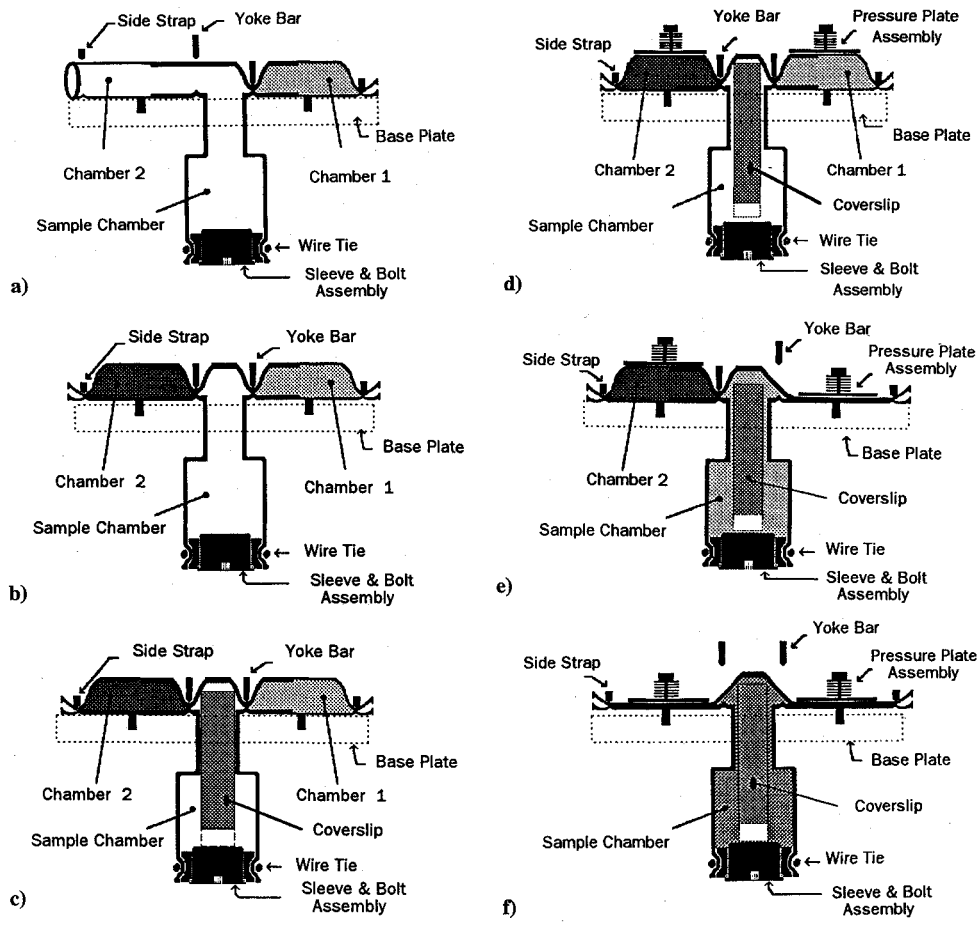

Fig. 2 Loading of test solution and biological samples into the Biomodule T tube: a) test solution loaded into chamber 1, b) stop solution loaded into chamber 2, c) coverslip with cells loaded into sample chamber, d) pressure plates attached to Biomodule, e) unlatched yoke bar initiating fluid transfer from chamber 1 to sample chamber, and f) unlatched yoke bar initiating fluid transfer from chamber 2 to sample chamber.

input and output capabilities, so that individual T tubes can eventually be instrumented with biosensors that will provide continuous data sampling during the course of an experiment. It is also important to recognize that the fluid transfer timelines of adjacent T tubes are totally under investigator control. Each solenoid controls the latching mechanism of adjacent T tubes and the subsequent transfer of fluids to their respective sample chambers. The activation of the Biomodule payload, on orbit, initiates the onboard computer to begin executing the experimenter-defined fluid addition timeline. Virtually any user-defined fluid transfer timeline can be accommodated. This is an extremely important point: since five to eight Biomodules are routinely flown on each mission, multiple investigators and divergent experimental protocols can be easily accommodated. This feature provides individual investigators with the flexibility to optimize their experimental timeline without any interference with other investigators. In addition, the versatility of the hardware enables mission managers to accommodate several investigators within a single payload on any given mission.

After the pressure-plate assembly mechanism is installed, a constant mechanical force or load is applied to the fluids and the elastic wall of the chamber (see Fig. 2d). When the onboard computer releases the yoke-bar latch mechanism, the mechanical force exerted by the pressure-plate assembly, working in conjunction with the elastic recoil of the chamber wall, forces the fluids from the side chamber of the T tube to the sample compartment (Fig. 2e). The movement of the yoke bar from the closed to the open position removes the physical barrier that had previously separated the two fluids. Since fluid entering the sample chamber is forced to move at a 90-deg angle, sufficient turbulence is generated to obtain nearly instantaneous fluid mixing. Although this mixing process produces some shear, it is insufficient to dislodge cells from the surface of the coverslip. In most cases, the initial fluid transfer serves to initiate a response, and the addition of the second fluid serves to stop the reaction (Fig. 2f). Although the transfer of fluid into the sample chamber produces a small increase in chamber pressure (2–3 psig), the permeable Silastic membrane gradually dissipates this pressure difference.

Containment Vessel Features

After several successful test flights aboard sounding rockets,[13] longer-duration missions were planned to further evaluate the complex biological responses that were observed during these brief flights (6–8 min). To provide a suitable experimental environment for extended-duration biological investigations and address important Space Transportation System (STS) safety issues, four important design objectives were initially identified. First and foremost, a mechanism must be developed to maintain a controlled experimental environment for labile biological samples over an extended period of time (3–14 days). Second, reliable procedures have to be developed to manage liquids and gases in applications where environmental control and containment are equally important. Third, if new hardware is required, it should be sufficiently versatile to accommodate an assortment of different experimental applications other than the Biomodule. Finally, any changes to the flight hardware must be user-friendly and provide the experimenter with the ability to rapidly load labile biological materials (provide late access) and then quickly recover the samples shortly after they are returned to the laboratory following the landing of the Orbiter (early recovery).

After careful consideration of the previously outlined objectives, a containment vessel was designed to interface with the NASA refrigeration–incubation Module (RIM) and the Biomodule payload. The resulting payload configuration, depicted in Fig. 3, was developed for the Space Shuttle. Eight Biomodules, containing a total of 64 biological samples, are packaged into the sealed container along with the required support hardware. The NASA RIM is flight certified, and it has been used extensively in applications requiring precise temperature control. The RIM is installed within a single middeck locker aboard the Orbiter, and it has been successfully utilized to maintain sensitive, late-access biological payloads.

The seamless metal containment vessel, depicted in Fig. 3, was designed to provide two levels of liquid and gas containment. The container and its removable covers feature internal ribbing that provides structural rigidity for the container as well as strength and support when the device is exposed to either under- or overpressurization. To optimize heat transfer, the Biomodule's attachment points to the containment vessel are positioned on the internal surface of the container in close proximity to the RIM thermoelectric devices. Removable internal bracing provides stable attachment points to the four side walls and a mounting surface for eight Biomodules. The internal side-wall structural supports can be removed and reconfigured to accommodate virtually any internal payload, thereby providing great versatility. The covers, passthroughs, and vent plug all contain two seals, which provide two levels of liquid and gas containment. The wide lid flange and double seal provide exceptional sealing capability when the container experiences pressure-induced flexing. The large removable covers also provide easy access to the interior of the container. One or more covers can be removed to accommodate sample loading. The covers are easily secured with standard fasteners to accommodate late access and early retrieval requirements. A vent plug is available to purge or precondition the payload atmosphere with a defined gaseous environment.

Confirmation of seal integrity prior to hardware turnover is accomplished by applying a vacuum to the internal space between the inner and outer seals. A test fixture is attached to a channel that connects the inner groove between the cover seals. This closeout vacuum test can be completed within minutes, and it provides certification that the container seals meet or exceed NASA safety guidelines. More importantly, however, this procedure is noninvasive in that it does not harm or alter the preconditioned atmosphere inside the experiment container. Therefore, critical biological environments can be maintained throughout the experimental period. The container is also configured with two sealed electric passthrough assemblies that provide multiple electrical service lines into and away from the container. The nonferromagnetic materials that have been used to construct the container also provide electromagnetic interference shielding.

The unique features ascribable to the containment vessel are outlined in Table 2.

Table 2 Containment-vessel features

Eight Biomodules or 64 samples per middeck locker.
Variable temperature regulation, 4–40°C, when used in conjunction with the RIM.
Two levels of gas and liquid containment.
Ability to maintain a stable internal pressure and gas composition within the container during extended periods of time. The payload environment is not affected during cabin depressurization.
Gas composition within the container can be altered to meet science requirements.
Internal structural elements can be reconfigured to accommodate other payloads.
The container is designed to accommodate late-access biological payloads.

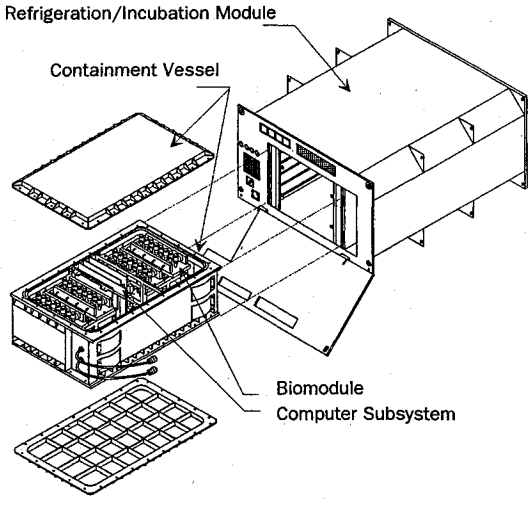

Fig. 3 Biomodule payload configuration.

Biomodule Life-Science Applications

An assortment of biological materials, including plant and animal cells as well as protein crystals, have flown in the Biomodule aboard sounding rockets. Preliminary reports outlining some of the initial experimental results have been published.[13,14]

Since the T-tube sample volumes are small, we have developed ancillary procedures to provide reliable quantitative measurements of limited amounts of biological sample. This methodology utilizes an encapsulation technology[15] to trap individual cells within agarose gel microdroplets as illustrated in Fig. 4. The agarose gel is sufficiently permeable to allow nutrients, gases, and waste products to diffuse into and away from the cells while retaining other high-molecular-weight molecules. In addition, the gel serves as a protective barrier, shielding the cells from physical damage during sample manipulation.

One important application of this technology is the use of antibodies to scavenge and trap important regulatory molecules.[16] Antibodies can be selected that specifically complex with important cell products such as hormones, enzymes, cell surface proteins, etc. These antibodies can be covalently linked to larger impermeable molecules and utilized as the basis for sensitive analytical assays that provide information on the functional properties of individual cells. When these antibody complexes are mixed with the agarose, they can be cosequestered with the cells during microdroplet formation (Fig. 4). As the cells secrete the desired product, it is bound to the antibody complexes within the agarose matrix and physically trapped within the microdroplet. At the conclusion of an incubation period, the cells are fixed and subsequently incubated with an appropriate fluorescent marker to detect the antibody-bound cellular product. The labeled cells are then evaluated by fluorescent-activated cell sorting (FACS). The intensity of the fluorescent label can be used to quantify the amount of product that is synthesized by a cell over some specified period of time. Therefore, this experimental approach could be employed to examine how individual cells function in a microgravity environment.

The gel microdroplet technology has proven to be a highly sensitive analytical tool when used in conjunction with FACS. We believe this experimental approach may be particularly useful for microgravity investigations, since small numbers of cells (10^4–10^6) can be used to generate a large number of data. For example, the recovery of only a few hundred cells at the end of a mission would still provide a valid statistical sample for FACS. Since fewer resources are required to culture smaller numbers of cells, this experimental approach has the potential to significantly increase the science yield.

To date, we have employed this technology to study β-galactosidase expression from bacterial colonies that were initially formed from an individual bacterium.[17] In other preliminary investigations, this methodology has been used to encapsulate and culture rat anterior pituitary cells. After a 72-h incubation period, the cells were fixed and stained with propidium iodide, a red nuclear stain, and an FITC-labeled antihormone antibody (yellow stain). Specific subpopulations of pituitary cells could be easily identified and counted with the fluorescent-activated cell sorter. These preliminary results demonstrate that this experimental approach can be employed to obtain detailed information on the functional properties of individual cells. This could be an important consideration, since some microgravity responses have been ascribed to specific changes within subpopulations of cells.[7,8,18]

Additional applications and benefits that are attributable to this technology are summarized in Table 3.

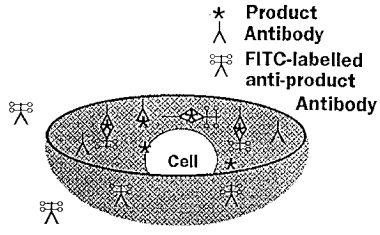

Fig. 4 Cross section of a cell within a gel microdroplet.

Table 3 Associated benefits of cell microencapsulation

Encapsulated cells are protected from damage during handling.
Specific physiological functions can be evaluated in subpopulations of cells.
Valid statistical results can be achieved with small numbers of cells.
FACS can be employed to identify and isolate unique cell types for further analysis.
Postflight sample analysis can be significantly automated and streamlined through the application of FACS technology.
The methodology can be easily adapted to microgravity gene expression experiments and postflight cell cloning strategies.

Conclusions

In this report, we describe new flight hardware and ancillary support methodology that has been developed with NASA sponsorship for microgravity life-science investigations. Our primary objective is to familiarize the scientific community with the operation and capabilities of this newly developed technology.

The Biomodule is the primary functional component of the versatile, computer-controlled fluid-mixing device described in this report. It is capable of supporting a broad range of microgravity life-science investigations. The Biomodules compact modular design enables it to be easily adapted to any launch-vehicle configuration. The hardware is designed to simultaneously accommodate 64 individual samples so that multiple investigators with diverse experimental designs can be easily accommodated.

The containment vessel, developed to house the Biomodule as well as other hardware applications, provides unprecedented environmental control for microgravity life-science investigations. Aboard the Shuttle, the Biomodule payload environment can be conditioned and controlled according to investigator-specified temperature, internal pressure, humidity, and gas composition requirements when it is used in conjunction with the containment vessel. This unique combination of hardware features provide the investigator with excellent containment and an acclimatized experimental environment in a single user-friendly device.

It is important to re-emphasize that this container provides two levels of liquid and gas containment. The internal parts of the container are easily accessed from different surfaces, there by providing great flexibility and optimal use of the internal space. The double seal provides high-quality containment of liquid and gas (leak rates below 1 part per 100,000 per hour) and a rapid, nondestructive and reliable test procedure to certify closure prior to late access turnover. The excellent sealing properties of the container allow the experimenter to specifically tailor the internal environment to address the needs of an experiment (e.g., provide a controlled internal pressure, humidity, gas composition, and temperature throughout a mission) while meeting the stringent safety concerns that are required for flight certification aboard the Space Shuttle (e.g., containment of toxic or hazardous materials). To our knowledge, no other existing flight hardware provides individual investigators with this level of flexibility and experimental control.

The microencapsulation methodology that is described has only been utilized, thus far, in ground-based studies. However, when this methodology is used in conjunction with FACS, powerful new experimental approaches involving molecular biology and genetics can be applied to test the molecular basis of the microgravity-induced responses that are currently being identified.

The comprehensive and user-friendly Biomodule and containment-vessel design features that have been described complement and extend NASA's life-science hardware capabilities. The availability of this hardware provides microgravity life-science investigators with additional options as they select hardware that is best suited to achieve their desired experimental objectives. Since the hardware is designed to accommodate multiple investigators, NASA program managers can simultaneously accommodate the scientific requirements of several investigators during each launch, thereby providing greater science yield at a lower cost.

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