Testing Prospects for Reliable Diatom Nanotechnology in Microgravity

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ABSTRACT

The worldwide effort to *grow* nanotechnology, rather than use lithography, focuses on diatoms, single cell eukaryotic algae with ornate silica shells, which can be replaced by oxides and ceramics, or reduced to elemental silicon, to create complex nanostructures with compositions of industrial and electronics importance. Diatoms produce an enormous variety of structures, some of which are microtubule dependent and perhaps sensitive to microgravity. The NASA Single Loop for Cell Culture (SLCC) for culturing and observing microorganisms permits inexpensive, low labor in-space experiments. We propose to send up to the International Space Station diatom cultures of the three diatom species whose genomes are being sequenced, plus the giant diatoms of Antarctica (up to 2 mm diameter for a single cell) and the unique colonial diatom, *Bacillaria paradoxa*. *Bacillaria* cells move against each other in partial synchrony, like a sliding deck of cards, by a microfluidics mechanism. Will normal diatoms have aberrant pattern and shape or motility compared to ground controls? The generation time is typically one day, so that many generations may be examined from one flight. Rapid, directed evolution may be possible running the SLCC as a compustat. The shell shapes and patterns are preserved in hard silica, so that the progress of normal and aberrant morphogenesis may be followed by drying samples on a moving filter paper "diatom tape recorder". With a biodiversity of 100,000 distinct species, diatom nanotechnology may offer a compact and portable nanotechnology toolkit for exploration anywhere.

Keywords: astrobiology, compustat, diatoms, feedback clinostat, microfluidics, microgravity, microtubules, nanotechnology

1. INTRODUCTION

A worldwide effort to grow nanotechnology[1,2], rather than use classical lithography, focuses on diatoms, single cell eukaryotic algae with ornate shells of glass (hydrated silica). Diatoms produce an enormous variety of structures (Figure 1). The silica can be replaced atom by atom[3,4,5] to form oxides and ceramics, or reduced to elemental silicon, creating complex nanostructures with compositions of industrial and electronic importance[1,6,7]. Diatoms grow anywhere there is sunlight and moisture. They form the green-brown scum in swimming pools and on surfaces in aquariums. They produce "20 to 25% of the world net primary production"[8], and could be used to regenerate oxygen in spacecraft.

We propose to send a variety of diatom cultures to the International Space Station (ISS) in exponential growth phase. The questions we are curious about include:

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1. Will normal diatoms have increased pattern and shape aberrations compared to ground controls?
2. Will auxospores (the spherical phase just after sexual reproduction) still be able to restore the normal vegetative morphology of a species after a few generations, as they do at 1 G [9,10]?
3. Can we test new theories of diatom morphogenesis[11,12,13,14,15] by seeing if they predict the correct microgravity results?
4. Can shape selection in a compustat[16] also work under microgravity?
5. Do motile pennate diatoms, especially those that are geotactic[17], alter their behavior in microgravity?

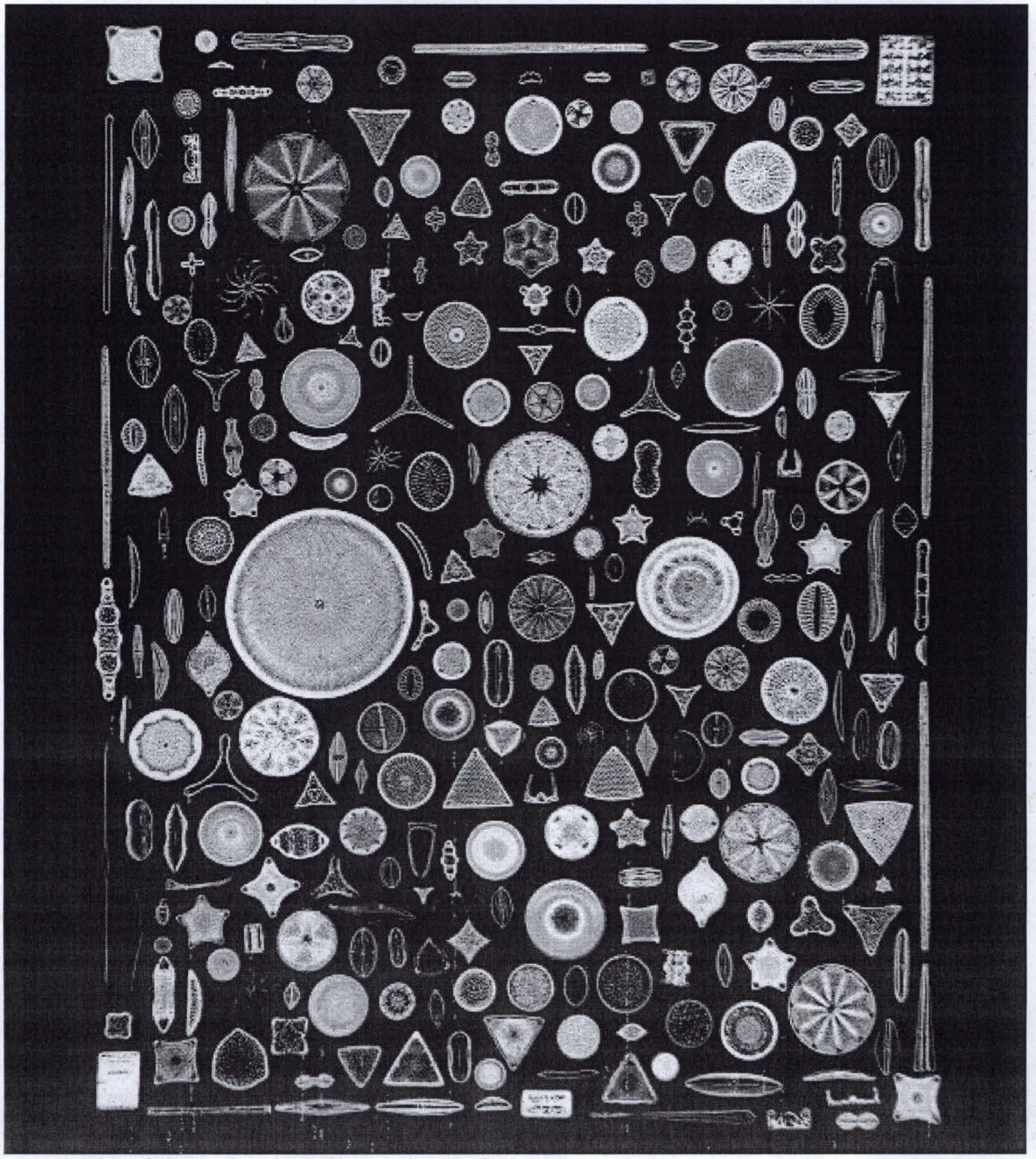

Figure 1. A variety of diatoms (by S.S.N.). The array is 1.78 x 2.30 mm.

2. BACKGROUND

> "You can look at something as simple as a diatom and realize that within its limited genome is the blueprint for creating three-dimensional structures in silicon that would be the envy of any engineer"[18].

Indeed, diatoms have been taken as models for macroscopic engineering and architectural structures[19,20], and they exhibit great mechanical strength[21,22]. The first paper on diatom nanotechnology[23,24], and our subsequent work[25,26], suggested that we could grow rather than lithograph nanoproducts. At the first workshop on the subject in 2003, there were 13 laboratories worldwide represented[1]. The view to space exploration came with a review[3] of "Star Trek replicator"-like work, in which atom for atom substitution changes silica to a variety of other industrially important materials[4,27,28]. Diatom shells may also be used as a mold or scaffold for other substances[6,29]. The conversion of diatom silica to silicon has already made new sensors possible[7,30], and of course opens up diatoms for use in 3D electronics that may compete well with or outflank MEMS[31,32,33].

Manufacturing of nanodevices may be necessary during interplanetary and interstellar space travel. Diatom cells vary from about 3 μm to 2500 μm in size[34], across the 100,000 species available on earth. A living library of all the diatoms on earth, one cell each, containing its unique genome, would occupy less than 1 cm^3, and so would be highly transportable. But will diatoms do what we want in space? The primary aim of this paper is to propose a program of collaborative research is to see if diatoms undergo normal morphogenesis in microgravity. Since the shaping of diatoms (not just mitotic spindle formation) is highly microtubule (MT) dependent[12,35,36,37,38,39,40,41,42], and MTs polymerize and aggregate differently in microgravity from their behavior at 1 G [43,44,45,46], we can anticipate that diatom morphogenesis might be disrupted, especially since other eukaryotic plant microtubules are disrupted in clinostats[47,48] (as are animal cells, such as osteoblasts[49]). The potential role of microtubules in diatom morphogenesis[11] has been simulated[12], though microgravity has yet to be taken into consideration.

A previous attempt to grow diatoms in space, which involved one of us (R.B.H.), unfortunately failed because the diatoms were sent dry to minimize launch effects[50,51,52,53], but the medium injector did not work.

Note that motility in diatoms also invokes the cytoskeleton, which is involved both in morphogenesis of the microfluidics chamber (the "raphe"), and in its operation during motility[54,55].

3. RELEVANCE TO SPACE LIFE

This is an interdisciplinary project, focusing on both the physical sciences (nanotechnology in space) and the life sciences (growth of nanotechnology in space). For the latter, at least, it contributes to fundamental, unsolved problems:

1. Diatoms solve the fundamental biological problem of morphogenesis within the confines of a single cell[3,11,12,25,26,56]. They thus present an opportunity to bridge the gap between genomics and the physics of morphogenesis[3,57]. It is difficult to decide *a priori* whether diatoms will show abnormalities of shell development in space. For example, in a literature review on animals in microgravity, it became apparent that frog embryos develop somewhat normally, while mammals do not seem to even get started, and to understand the difference[58], empirical studies are required.
2. We plan to include diatoms whose DNA is being sequenced, i.e., "standard organisms". The use of standard organisms has not been emphasized in space life research, resulting in heterogeneous data that cannot easily be compared and built upon[58]. Diatoms will be grown under microgravity, dried, scored for normal/abnormal growth and growth rate, and aberrant cells tested for ability to recover form in subsequent generations. Some cells will be dried and stored live for later investigation of possible mutations or somatic inheritance[59,60].
3. Diatoms are phototactic[61,62] and geotactic[17]. Cells of the unique motile colonial species *Bacillaria paradoxa* slide back and forth against one another in partial synchrony[63], providing an opportunity to investigate gravity dependence of cell-cell interactions. An advantage of diatoms over most other motile eukaryotic cells[64,65,66,67] is that they do not change shape during motility, so that the motor mechanism and its control[54] can be studied

without that complication.

4. CHOICE OF DIATOMS

We will start with all three genome diatom species being sequenced:

1. *Phaeodactylum tricornutum,* a motile pennate[68,69,70] (Figure 2).
2. *Fragilariopsis cylindrus,* a motile pennate with a marginal raphe[69,71,72,73,74] (Figure 3).
3. *Thalassiosira pseudonana*, a centric[72,69,73] (Figure 4).

There are 100 to 200 papers on each species in the scientific literature, so there is a substantial intellectual community interested and involved. The three species provide a plausible sampling of kinds of diatom morphologies, though there are of course many more available.

Diatoms are the most extremophilic eukaryotes on Earth[75]. For example, diatoms are the dominant eukaryotic life forms in sea ice, glaciers and permafrost but they also occur in geysers and hot springs. Diatoms are found in acidic lakes and in consortia with cyanobacteria they are often the primary producers in hypersaline and hyperalkaline lakes and lagoons. The diatoms we suggest for this research effort include the extremely well studied psychrophilic diatom *Fragilariopsis cylindrus* (Grunow) Krieger[76]. This diatom is abundant in the sea ice of both the Arctic and the Antarctic. *Fragilariopsis cylindrus* is typically restricted to very cold water (<1°C). It is an obligate psychrophile and grows very slowly at temperatures above 5°C. *Fragilariopsis cylindrus* possesses very important ice binding proteins[77], which whole genome studies have shown to be absent in the mesophilic diatoms selected for this study -- *Phaeodactylum tricornutum* and *Thalassiosira pseudonana.* The diatom *Thalassiosira pseudonana* is a very well studied cosmopolitan marine centric. It is of particular interest for this research and considerable work has already been done on the role played by silicon transporter proteins in silicon metabolism during the cell cycle[78].

5. GROUND EXPERIMENTS

5.1 Chemostat culture

We might best begin with long-term culture of the diatom species in chemostats[79,80,81,82,83,84,85], to determine the baseline morphology, variation[86,87,88,89,90,91,92,93,94,95,96], and spontaneous formation of aberrant forms[97]. We need to compare batch culture and clonal[86,97,98,99,100,101,102] subculturing with chemostat culture. If aberrant forms grow more slowly, then chemostat culturing may preserve normal diatom morphology better.

We anticipate some problems keeping the motile, and therefore adherent *Phaeodactylum tricornutum*[103] and *Fragilariopsis cylindrus* from coating the chemostat wall, but a stirring ball, designed to minimize gas transport gradients in the medium (so that we focus on microgravity effects within the diatom cell rather than in the medium[104]), could also double as a scrub brush[105]. In this case, we will have to do ground assessment on how to minimize the number of broken cells and how to distinguish broken cells from aberrant cells.

5.2 Quality control via FACS

The question of quality control in diatom nanotechnology is yet to be addressed. We might begin by characterizing the spontaneous 1G aberration rate and forms, and their growth rates, in selected species. A FACS machine (Fluorescent Activated Cell Sorter, or flow cytometer), probably run in the light scattering mode[106,107], would provide massive statistics on cell variation in clonal cultures. As FACS has not previously been used for diatom morphological variations and aberrations, we will have to experiment to see if it will be a valuable adjunct to direct visual inspection, and how it correlates with visual criteria, perhaps best obtained by SEM (scanning electron microscopy). The FACS machine provides not only a measure of reproductive precision of diatom shells, but also permits the sorting out, i.e., physical separation, of those that do and do not meet specified criteria.

Diatom parts that are currently separable include valves, nascent valves, girdle bands and auxospore scales[108]. It might be valuable to find ways to break apart diatom shells into smaller units, such as ribs, 3D pore units, intact raphe units, spines, etc., before or after cleaning out cell contents with detergent[108,109]. Methods might include partial HF digestion[110], ultrasound, cryogenic ballistic smashing, or crushing [21,22,111,112]. FACS might help sort out these parts while providing quality control.

5.3 Compustat

The compustat works by examining images of all the diatoms in a chamber, and destroying those that are farthest from a prespecified ideal form, using a UV (ultraviolet) or laser pulse microbeam[16]. Those cells remaining proliferate, as in a chemostat. Mutagens may be incorporated to speed this directed evolution. One design for a compustat would be an iterative flow cytometer, in which selected cells are recycled after being allowed to proliferate. No one has yet built a compustat. We need to ascertain how far diatoms can be pushed by artificial selection towards making shapes we dictate.

5.4 Clinostat

No clinostat experiments have been reported on diatoms, despite their known geotropism[17,55]. Clinostat experiments with the motile, adherent diatom *Phaeodactylum tricornutum* would be worth conducting on the ground, simply by rotating vertically mounted microscope slides under medium, and scoring them for aberrant cells compared to unrotated slides. We could also try growing them embedded in a gel, to control their orientation to gravity. If an effect either on morphology or motility is found, then a new class of clinostats could be considered (conceived by B.E.L.), called "feedback clinostats": the rotation is continuously adjusted to compensate for the movement of some feature of a single cell, such as its direction of movement, instead of using a fixed program of rotation.

5.5 Diatom tape recorder

The usual procedure for recording the progress of microorganisms would be to sample and preserve aliquots of their growth medium. We propose instead continuous sampling by passing some of the medium through a moving filter tape, providing a continuous record of dried diatoms. The dessicated diatoms would be in suspended animation, from which some (but not all[113,114]) can be recovered[114,115,116,117,118,119,120,121]. This "diatom tape recorder" permits thousands of samples to be taken continuously or intermittently, without subculturing, and may permit reversible preservation of diatom samples without astronaut intervention. Some experimenting with hydroscopic materials presoaked into the filter might extend the range of diatoms that could be dessicated and resuscitated.

The filter tapes would be polycarbonate or polyester membranes (Sterlitech Corporation, Kent, WA) that are transparent when wet, and thus suitable for substrates for light and other forms of microscopy. They have precise pores that are the etched tracks of alpha particles, and with pore sizes down to 0.05 μm, diatoms can be reliably retained, without getting stuck in the pores.

6. ON BOARD EXPERIMENTS

We suggest using apparatus based on NASA's Cell Culture Unit (CCU)[122] (Figure 5)[104,123], which was designed for use onboard the ISS. This apparatus does much of what we want, though it may have to be modified for chemostat and compustat culture.

The hardware consisted of:

- the Cell Specimen Environment Assembly, which holds up to eighteen Cell Specimen Chambers (CSCs, each with 3 to 30 ml capacity), permits up to 60 samples to be drawn (or injected) and stored under preprogrammed or ground control, and provides growth medium perfusion, additive delivery, and heat and gas exchange for each chamber individually;

- the Electronics Assembly, which contains all computer and signal conditioning;
- the Video Microscopy Subsystem, which provides 40x and 200x optical magnification views of the specimen cultures to the crew or video downlink to scientists on the ground;
- the Structural Containment Assembly, which supports all the other assemblies and provides interface mounting with the Shuttle and ISS host systems"[122].

Temperatures down to 4°C can be maintained[123], so that psychrophilic diatoms could be cultured near their temperature optima. Before the project was completed, the requirements were modified and the CCU was replaced by 12 simpler units (Figure 6) that take single cultures: 4 to fly, 4 for ground controls, and 4 for backups[124,125]. These Single Loop for Cell Culture (SLCCs) were delivered to NASA Ames in June, 2006, but have not been flown yet. Free swimming *Euglena* were cultured in a closed loop[123], which is not equivalent to a chemostat, since only small portions are removed for sampling. The cells were confined to the growth chamber by a porous membrane[104].

Illumination would be with cool-white fluorescent lamps (3500-4000 lux)[126] or bright white LEDs. This system easily comes in under low cost, volume, mass, power and astronaut labor constraints. Furthermore, it can stay in the ISS indefinitely, as nothing has to be sent up after initial launch except new filter tapes, salt (from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, which also supplies type cultures: http://ccmp.bigelow.org/) and dye for the medium (water soluble India ink, which can provide some timing markers) and diatom innoculi in fresh chemostat chambers. Nothing has to be returned to earth except the filters. Because each filter records a whole experiment, no morphological data has to be transmitted to earth during an experiment. Of course, for motility experiments, transmission of visual data would be available.

The filtered water is a harmless, dilute salt solution, which could feed directly into the International Space Station Environmental Control & Life Support System[127,128,129]. Additional medium could be made up from on board water supplies and packets of salts.

One advantage of the SLCCs is that the magnetic stirrers in them were tested for mixing of the medium by empirical and CFD (Computational Fluid Dynamics) software approaches[104]. The flow of the medium in microgravity could be further investigated via ComFlo, a CFD program that was developed for simulating the motion of rocket propellant in microgravity during spacecraft acceleration, and its effect on spacecraft motion[130,131,132,133,134].

7. POST-FLIGHT ANALYSIS

The post-flight analysis would be mostly visual comparison of the ground controls and diatoms on the returned filters or in sample chambers, using light microscopy[75], scanning electron microscopy (SEM)[9], and Secondary Ion Mass Spectrometry (SIMS)[135,136]. The methods would include those we have published for microorganisms that may be in meteorites[137].

If aberrant forms are found amongst the microgravity exposed diatoms, those examined by nonlethal light microscopy will be used to start cultures to see if the aberrations propagate or if normal morphology is restored. The time course of their progress can again be followed with a ground-based copy of the diatom filter tape recorder.

8. CONCLUSION

The relevance to of diatom nanotechnology space research and technology includes:

1. the very first attempt towards growing nanotechnology in space;
2. emphasis on standard organisms for research in space;
3. understanding of the fundamentals of morphogenesis and cell motility in microgravity;
4. first attempt at directed evolution of diatoms towards producing specified structures;
5. assembly of a nanotech kit for growing a vast array of nanotechnology components.

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Figure 2: "Light Micrograph of *Phaeodactylum tricornutum*. This pennate diatom is the 'lab rat' of diatoms, and its genome sequence is currently being determined. (Image courtesy of Alessandra de Martino and Chris Bowler, Stazione Zoologica and Ecole Normale Supérieure.)"[24].

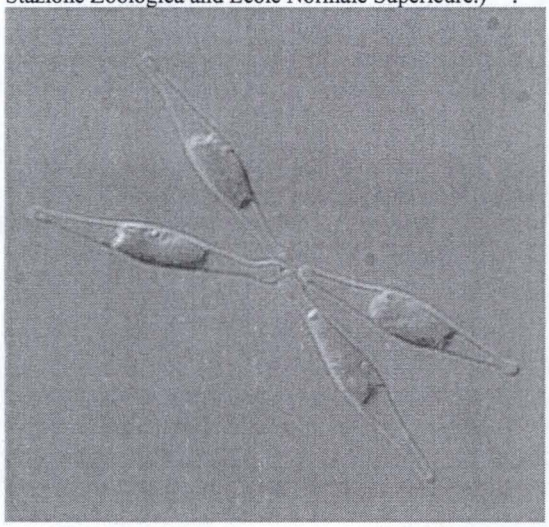


Figure 3: *Fragilariopsis cylindrus,* shown in "valve" view SEM (scanning electron micrograph)[71].

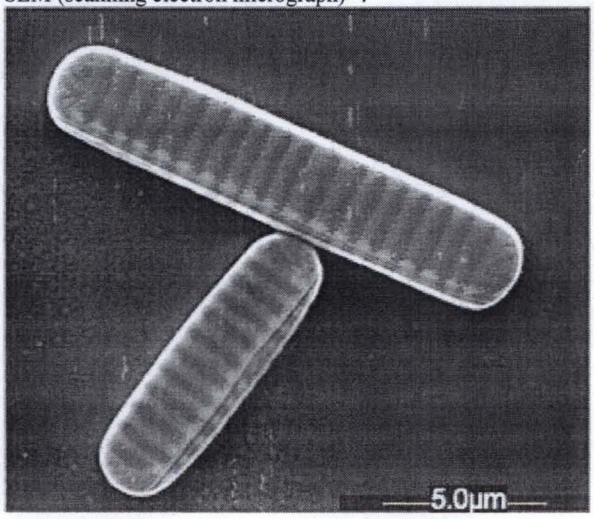


Figure 4: Scanning electron micrograph of *Thalassiosira pseudonana*[138].

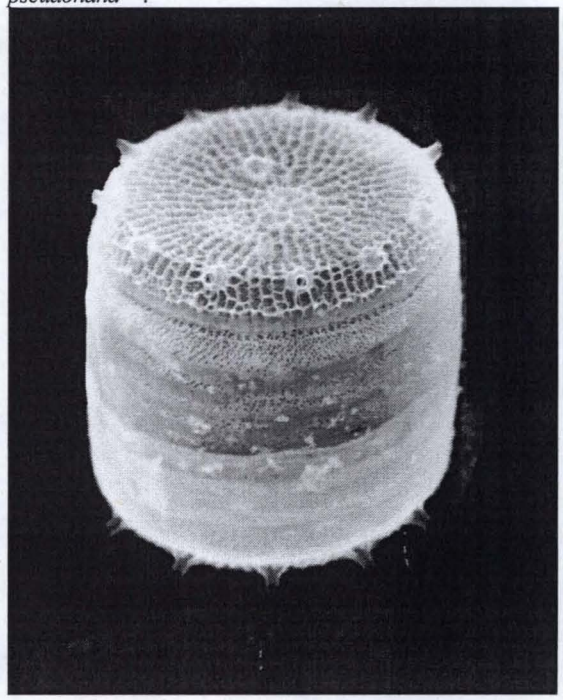


Figure 5: NASA's ISS Cell Culture Unit (CCU)[122].

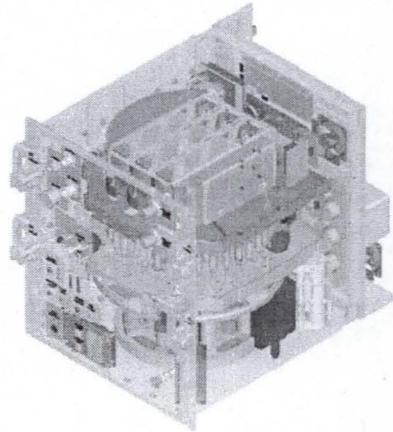
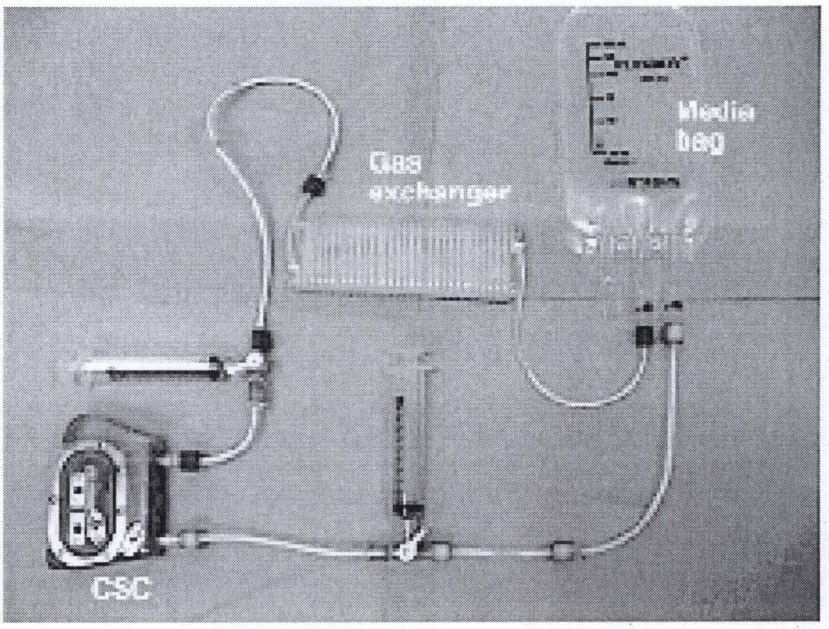
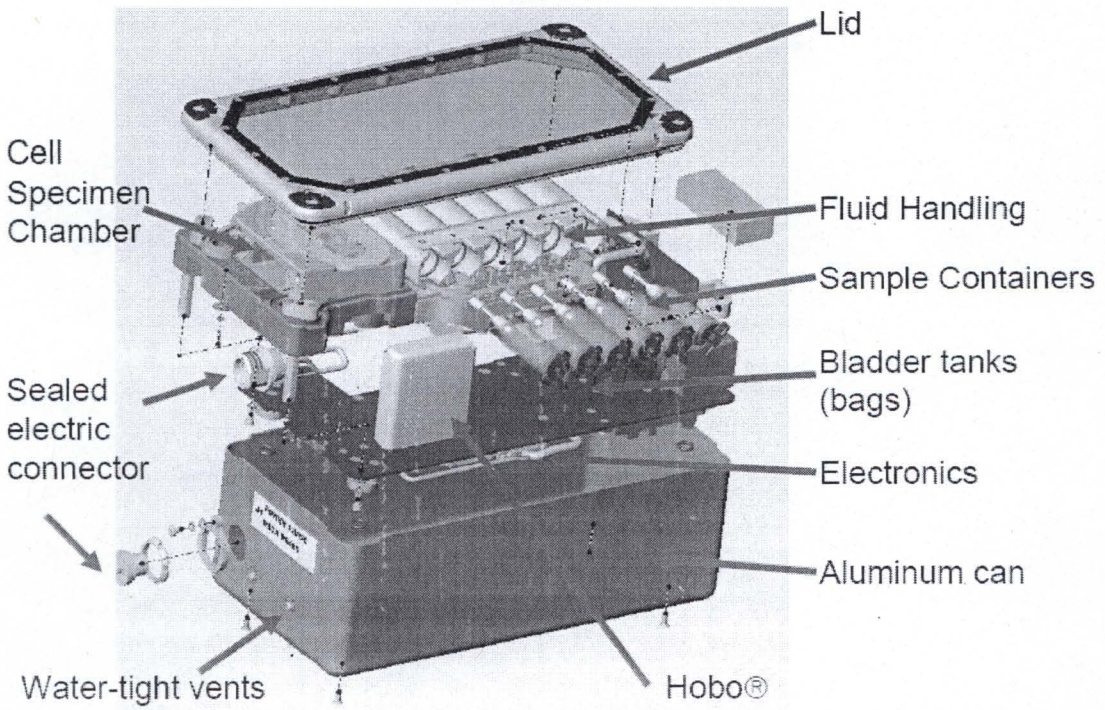


Figure 6: SLCC, Single Loop for Cell Culture: "The single loop consists of a CSC [Cell Specimen Chamber], media bag, gas exchanger, and sample and injection syringes (pump, stir bar motor and controller not shown)"[123]. The payload characteristics are: size: 3.4" x 5" x 10" (2.8 liters); power: each SLCC unit requires 2 Watt (165 mA at 12 VDC) steady state power, with up to 3.3 Watts of peak power when additional pumps and valves are operating; weight: 5.5lbs (2.5 kg); automation: it is automated, except at the end of the test, crew member is needed to help for last sample drying, takes less than half an hour. An exploded view of the SLCC is also shown.





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