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Design Principles for Microfluidic Biomedical Diagnostics in Space

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1. Introduction

The human body adapts to the space environment in a number of direct and indirect ways. With the near-removal of gravitational forces, body fluid tends to move headwards from the lower extremities. As the body adapts to this changed distribution of fluid, plasma volume in the blood decreases within days. This change increases the relative concentration of red blood cells in the blood, which may be a factor in the observed decrease in red blood cell generation. (For discussion of this and similar issues, see, e.g., (Blaber et al., 2010)). Newly modified concentrations of various dissolved gases, hormones, electrolytes, and other substances induce a cascade of inter-woven effects. Other direct effects of space include conformational changes in compliant tissue, including the shape of the heart. Muscles tend to atrophy without the challenge of gravitational loading. More troubling, key regions in the load-bearing skeleton, such as the hip and lumbar spine, can lose mass at a rate of 1.5-2.5% per month, at least for several months. Aside from the loss of gravitational loading, other environmental factors include radiation exposure (Akopova et al., 2005), altered light/dark cycles, and the psychological stress of living in a confined space in a dangerous environment with only a few people for months at a time. Biomedical research continues to expand our knowledge base and provide insights into the short- and long-term effects of spaceflight on the human body. Space medicine is concerned with the more immediate needs of astronaut patients who are living in low earth orbit right now. Both of these tasks require biodiagnostic tools that give meaningful information.

The retirement of the Space Shuttle removes the primary avenue for returning astronaut blood samples to earth for lab analysis. This requires a shift in focus from ground-based analysis of space-exposed samples to on-orbit analysis. While this is a significant challenge, it also provides an opportunity to use the International Space Station (ISS) to develop next-generation medical diagnostics for space. For long-duration spaceflight, we know that the crew will have to operate at an unprecedented level of autonomy. The need for compact, efficient, reliable, adaptable diagnostics will be critical for maintaining the health of the crew and their environment. The ISS can be used as a proving ground for these emerging technologies so that we can refine these tools before the need becomes urgent.

The demands placed on onboard diagnostics are not simple to meet. Minimal resources for power, storage, excess volume and mass are available. Assume that the entire medical supply kit for long-duration spaceflight will be roughly the size of a shoebox. Little or no supply chain is to be expected. Devices and their supporting reagents and additives must remain viable for

several years. They must operate safely and reliably in an extreme envi-ronment. We would like to have testing capabilities that could respond to current needs as well as to evolving priorities. For blood analysis, we would like to perform routine chemis-try panels and cell counts as well as examine an array of biomarkers (some as yet unknown), which would aid in detecting radiative damage and assessing changes in bone, immune, cardiovascular, neurological, renal, and other functions. Aside from blood analysis, urine or saliva could provide useful diagnostic data while reducing invasiveness to the astronaut. Consequently, we would like to design towards a device that could accept other sample types including cell cultures, animal blood and urine, and environmental samples like potable water.

On earth, the Holy Grail for portable medical diagnostics is the development of a self-contained, robust, general-purpose assay system for analysis of bodily or environmental fluids. While this remains the goal, most research today focuses on bits and pieces of such a device, such as separation processes, onboard miniaturized sensors, or the development of highly specialized assays, e.g., detection of specific cancers. There are fewer investigations that focus on integration of sample introduction, processing, and detection with wide-ranging capability. But the pace of such development has progressed rapidly over the last decade. Most systems rely heavily on disposable components, which are a luxury for a spacebound tool. Devices that depend on bulky external infrastructure for optics, flow control or application of magnetic, electric, or optical fields are also ill-suited for space. Dynamic reconfiguration of device capabilities to accommodate different assays or sample types can be achieved with flow control and/or biochip-based reconfiguration, but these modular concepts remain more of an interesting oddity at present than a regularly employed strategy in biochip development. Complete integration, miniaturization, adaptability and breadth of capability are all essential features of a spaceworthy device.

On the commercial side, there are a handful of point-of-care devices that provide general-ized blood analysis. None of these devices span the breadth of assays specified by the most current medical requirements (International Space Station Program, 2004). For a discussion, see (Nelson & Chait, 2010). For these devices, the design paradigm is a suite of disposable cartridges, coupled with a reusable reader. But for space travelers on extended missions, the volume and mass of the disposable components for these commercial systems are prohibitively large. Moreover, the shelf life is grossly inadequate.

For the past decade, NASA has invested in the development of next-generation medical diagnostics. In this chapter, we consider the benefit of squeezing down the resource requirements by limiting the use of disposable components. Instead, we emphasize the reuse of the microfluidic and detection infrastructure if cross-contamination can be avoided. The basic technology exists today to build a reusable microscale lab analysis tool that is versatile, resource-conscious, and miniaturized to an extent that beats the commercial devices by a very large margin. The primary obstacles are in development, specifically, the integration of all the functional components into a single device, the capacity for massive multiplexing and the broad availability of appropriate assays. Many excellent reviews on microfluidics are available in the literature, including (Arora et al., 2010; Bhagat et al., 2010; Chan, 2009; Chang & Yang, 2007; Cho et al., 2010; De Volder & Reynaerts, 2010; Di Carlo, 2009; Gossett et al., 2010; Huh et al., 2009; Hwang & Park, 2011; Kim & Ligler, 2010; Kist & Mandaji, 2004; Kuswandi et al., 2007; Lange et al., 2008; Lenshof & Laurell, 2010; Mogensen & Kutter, 2009; Mukhopadhyay, 2005; Pamme, 2006; Pamme, 2007; Salieb-Beugelaar et al., 2010; Sun & Morgan, 2010). Consequently, in this work, we will focus on the new directions that will reduce resource consumption, improve adaptability and expand breadth while increasing diagnostic value within the framework of a single device.

2. Requirements for spacebound devices

Diagnostics in space must be stingy with resources, such as volume, mass, power and reagent consumption. Ideally, biomedical devices should be highly adaptable to meet evolving needs of space medicine, biomedical research, plant, cell and animal biology, and environmental monitoring. Devices and their supporting reagents and additives must sustain performance during a multi-year lifetime in a low-gravity environment, characterized by radiation, low humidity and the lack of refrigeration. Efforts to reduce additives, expand capability, amplify ruggedness and simplify controls will ultimately be beneficial for all next-generation medical devices, whether for use on earth or in space.

2.1 Resource consumption

In 2010, a prototype microfluidic device purified water and mixed it with salt crystals on orbit to deliver medical-grade saline solution in an approach described by (Niederhaus et al., 2008). This technique could maintain the availability of saline for medical or lab use on the Space Station, while eliminating the need to consume limited storage space in resupply vehicles. But in general, additives, including reagents, buffers, and clean water, are very limited. No dedicated hardware can be expected for cleaning or storage. Every pipette, lancet, cleansing wipe, and other supplies used for device operation or maintenance must be considered as part of an assay system's resource "load" and scrutinized for potential savings. From the standpoint of resource efficiency, the microdevices fabricated by embedding polymer microchannels on paper by Whitesides and co-workers are noteworthy (Martinez et al., 2010). A droplet of blood is applied to a snippet of paper, which uses capillary action to draw the sample through the device to colorimetric sensor pads. The overall system is extremely small, requires no power or other infrastructure for qualitative measurement, and can easily perform several blood tests simultaneously. Expanding its capabilities to dozens or hundreds of tests per sample is a major challenge, but this direction is progressing through the use of multi-layer microsystems (Martinez et al., 2010). However, this approach is unlikely to provide cell counts without major redesign.

One fundamental design choice is the use of a disposable cartridge, which accepts the sample and contains the microfluidic network necessary for processing the sample, versus a reusable system, which would ideally reuse all of the microfluidic infrastructure. In the latter case, only the sample itself and supporting additives would remain as biological waste. Those additives include the fluids required for flushing or cleaning the reusable device to remove all detectable traces of the sample and reagents. In contrast, the disposable cartridges encapsulate the biosample within its borders, simplifying the process of disposal while potentially eliminating the risk of cross-contamination. And, certainly, the disposable components could be designed to be more resource-conscious than current commercial designs. But even so, systems based on disposable cartridges will likely require more upmass for resupply than a reusable system would require. ("Upmass" is a term used to describe the mass occupied by something placed in a spacebound vehicle.) Close examination of the resources required by the device, the availability of flushing fluids and of upmass, the tolerance for risk, and the diagnostics requirements of the mission will determine the best approach for any specific space mission.

A device designed for reusability can be more easily re-engineered to operate with a disposable, bioencapsulating cartridge than vice versa. Consequently, in this work, we will examine the less studied problem of a reusable biomedical analyzer.

There are many avenues for scaling down a biodiagnostic system's footprint:

- *Reduce the scale at which the device operates*. Operating volumes on the scale of several hundred nL have been proven. The need for effective pre-filtering is however essential.
- *Include onboard sensors and fluidics*. Optical and electrochemical detection are at this point reasonably mature. Fluidic components such as micropumps and valving can be integrated with electronics for speedy communication and improved ruggedness.
- *Exploit shared resources*, such as a PC for signal processing and display. USB ports can also be used to supply power that is adequate for well-designed systems.
- *Scale back the size of the disposable portion*. Examples include a disposable insert for sample retrieval following separation by capillary electrophoresis (Mohanty et al., 2006) or portions of a microperistaltic pump (Liu et al., 2003).
- *Incorporate modularity*, introduced into the system virtually through dynamic programmability, or physically by using quick disconnects or swapping out chips for different sample types or detection strategies (see §2.2).
- *Use systems that can perform massive multiplexing*, i.e., process dozens to hundreds of diagnostic tests from a single sample (see §2.2 and §4.2).

2.2 Flexibility, adaptability and modularity

For space biodiagnostics, we would like to perform garden-variety blood and urine analysis, as well as more specialized tests. All testing will involve counting particulates, such as cell, platelet, bacterial, and crystal counts, as well as detecting concentrations of dissolved substances in the sample, including gases, electrolytes, small molecules, and a range of large and small proteins (see §4.2). Due to the sheer number of assays to be processed, one key requirement will be the capacity for massive multiplexing on an individual sample. ("Multiplexing" simply refers to the capability of performing multiple measurements on the same sample.) The multiplexing should avoid "hard coding" an assay suite to the system to permit adaptability. Finally, we would like to extend the range of sample types to more broadly address the needs of physiological, biological and environmental analysis.

Mix-and-match modules with simple connection strategies are one way to embed flexibility into a resource-conscious assay system. In one example, the development of "Zero Insertion Force" sockets allowed reconfiguration of the fluidic and electrical connectivity with up to 60 independent electrical connections joined to a variety of microfluidic systems (Dalton & Kaler, 2007). For space diagnostics, a module for cell separation and counting could be coupled to a serum analysis module. The latter could be designed to operate on nL-scale volumes of well-filtered fluid. The blood pre-processor for cell separation and analysis could then be swapped out with a urine pre-processing stage, which would separate out solids content, while sending well-filtered urine on to the nanoscale detection device.

Alternatively, dynamic flow control could be used to route the sample through different pathways to appropriate processing stations onboard a single chip. The basic principles of programmability that have become so integral to information technology are available for fluidic networks as well. In this case, the assay suite and the flow path can be specified at runtime, which can be effective at conserving reagents and streamlining operations. The rudiments of a Java interface were developed for operations on arbitrary portions of one biochip design, including metering, transporting, mixing, and purging (Urbanski et al., 2006). A device for studying protein precipitation included a Labview-driven system for quickly creating complex mixtures using 32 stock reagents in a 5 nL reactor (Hansen et al., 2004). The metering precision of 83.4± 0.6 pL ensured minimal reagent consumption. These approaches could add significant capability to a resource-conscious, adaptable biodiagnostic system.

Both of the strategies discussed above assume that reconfiguration requires the sample to access different sets of fluidic pathways, such as various reagent reservoirs, mixing devices, incubation chambers and/or detection stages. In §4.2, we will discuss other approaches to massive multiplexing through assay design.

2.3 Lifetime

Devices and their supporting consumables must remain viable in a space habitat for years in an environment characterized by radiation exposure and low humidity, without dedicated hardware for sterilization or guaranteed access to refrigerated storage. This is a significantly higher bar than the requirements on shelf life for earthbound devices. Moreover, little or no supply chain will exist for maintenance or replenishment. Therefore, NASA must find a solution with the right combination of physical and biochemistry, device and sensor design, and materials and fabrication choices to operate under the demanding conditions of long-duration spaceflight.

Like most commercial systems, the widely used i-STAT blood analyzer employs single-use assay cartridges. Its cartridges are rated by the manufacturer at a 4-6 month shelf life when refrigerated at 2-8˚C. NASA researchers found that some of the more common assays for blood chemistry retained their effectiveness for up to 12 months (Smith et al., 2004). No such testing is available for the single-assay cTNl cartridge, which detects troponin. This is problematic since the biochemical reagents in immunoassays are less stable over time than chemical reagents. Additional packaging to reduce the effects of gas diffusion and radiation exposure is likely to be the maximum effort that NASA could reasonably consider to extend the lifetime of commercial devices. See §4.2 for further discussion on assay design.

Few studies explore the impact of long-term exposure to space radiation on shelf life. One of the only studies to address this issue is considered in a preliminary fashion by (Fernandez-Calvo et al., 2006). High doses of low-energy gamma radiation were not found to be significantly deleterious on antibody activity, which was contained in stabilizing solutions. However, the duration of exposure was not reported in this work, and shelf life was not addressed. Furthermore, the study does not address the type of radiation of most concern, i.e., that of large energetic particles, such as high-energy protons and neutrons emitted in solar flares. More work in this area would be valuable.

Biochips must maintain geometric stability over their lifetime in space. Polymer chips are easy to manufacture, but are softer than silicon and glass, and may be more vulnerable in this regard. Cleaning and maintenance functions also impact this aspect of chip lifetime, particularly if disassembly is involved (Xie et al., 2005). Another concern is the degradation of seals, coatings, and sensing components, such as electrodes. Shelf life is certainly an issue for microscale electrodes (Shen et al., 2007; Zhang et al., 2007). Degradation could also affect overall containment in addition to sensing performance, and it could introduce fouling contaminants into the microfluidic network. The latter is of most concern when the contaminant is introduced downstream of filtration. One solution is to use techniques that isolate degradable sensors like electrodes from contact with fluids in the device (Nikitin et al., 2005).

Operational lifetime will be a function of the underlying (bio)chemistry, the biochip design, and the environment in which the sensor operates. In the literature on biochips, operational lifetime is occasionally reported, particularly for reusable immunoassays with expensive antigens or other biological recognition elements, since binding kinetics can degrade with cycling. Some studies report adequate function with some reusability (Chen et al., 2003; Yuan et al., 2007), depending on storage conditions. In spaceflight, devices must be stable for several years with hundreds to thousands of uses.

2.4 Gravitational independence

Mars exploration requirements prompted the development of biochip-based solutions for detection of organic acids using capillary electrophoresis (Skelley et al., 2005), which integrated power, pneumatic control and optics. Some microdiagnostics for biology have been tested in specially outfitted aircraft in parabolic flight campaigns. In this reduced gravity environment, a sequence of roughly 40 parabolic trajectories are traversed. At the peak of each parabola, 20-25 seconds of microgravity can be attained. Such testing provides insight into behavior on the Space Station, in which the net quasisteady acceleration is typically on the order of a few μg (where 1 μg=1x10-6g), although there can be much larger vibrational and transient disturbances (Nelson, 1994). Microfluidic devices that have undergone parabolic flight testing include electrophoretic separation of amino acids in an aqueous environment (Culbertson et al., 2005), an immunoabsorbent assay (Maule et al., 2003), and more recently, a blood analyzer on a reusable microfluidic platform (Chan et al., 2011).

We should not expect large variations in biochemistry (e.g., the biokinetic constants that describe binding processes) over short periods in reduced g. However, we should anticipate altered heat and mass transport due to differences in fluid behavior. Gravity and other accelerations act on density differences to drive processes such as sedimentation, bubble migration and natural (buoyancy-driven) convection. As we decrease the size of fluidic channels, we create a system with an increased surface-to-volume ratio, which in turn changes the relative importance of the forces that drive fluid transport. Macroscale systems, such as separation of plasma from whole blood through centrifugation, are governed by volumetric forces such as gravity and centrifugal acceleration acting through density variation to produce the separation. In microfluidics, surface forces, such as friction and surface tension, become more important. Nevertheless, we must also consider the altered competition of intermolecular forces leading to observed binding kinetics between neighboring surfaces and the bulk liquids in microfluidics devices. Both molecular orientation changes and direct molecular attraction/repulsion through surface coatings could potentially influence overall assay results if bulk transport is affected in microgravity.

In microgravity, particles are less likely to sediment in any static system, but bubble management can become more challenging. Processes that require large liquid/vapor interfaces to generate bulk flows in liquid will operate in an even less intuitive manner in microgravity. In space, surface-tension forces dominate liquid/vapor interface behavior without the application of other forces, creating blobs of liquid in unconfined space, or fully wetted surfaces with an interior bubble in enclosures. To be prudent, processes that can create bubbles must be analyzed for microgravity operation before use in space. In some cases, bubble generation is fundamental to the technique (Satoh et al., 2007; Furdui et al., 2003) and in other cases, bubbles can be an unintended consequence, e.g., of hydrolysis (Lancaster et al., 2005). Metabolic processes or biochemical reactions can create dissolved gases. Temperature and/or pressure variation can thermodynamically drive dissolved gases out of solution. Over time, liquid evaporation and gas diffusion through seals or porous materials can cause gaseous pockets to form in a liquid-filled device. Wherever there is a discontinuity in the fluid path, the potential for bubble generation exists. The location at which the sample meets the microfluidic device is one such juncture.

These considerations are of interest because bubbles can effectively clog the pathways of a microfluidic device and/or interfere with the detection process. The consequences are more serious for a device in space because bubbles are less inclined to migrate to specific

locations, such as the top of the device, for easy removal. One strategy is to prime the biochip system on earth so that all bubbles are removed before leaving for space. For short-term usage in carefully designed systems, this may provide adequate bubble control, but for long-duration flight, it is probably insufficient. Application of a large pressure gradient is a brute-force method to clear the fluidic channels of bubbles. As the channel dimensions decrease in size, the pressure gradients needed to drive bubbles out of the device increases, particularly for microfluidic channels with torturous geometry. A recent parabolic flight experiment demonstrated that a net pressure difference of about 10 kPa was sufficient to clear even large bubbles of air through channels with a cross-section of 200x120 μm^2 (Chan et al., 2011), although this device was intentionally designed to avoid channel features that could become bubble traps. Wetting properties in microchannels can be affected by surfactants, which can reduce the resistance to flow caused by bubbles (Fuerstman et al., 2007). Another strategy is to propel bubbles out of the system through the use of gradients in wetting properties of the walls, created through active control of an electrical field (§3.2.1). For our purposes, unless a device employs electrowetting for other purposes, it is not worth including it if application of pressure is adequate.

While the density ratio of most vapors to aqueous solutions is on the order of 1000:1, the density variation between typical biological liquids and particles is just a few percent, so the effect of gravity is less significant than for bubbly liquids. However, even at the scale of 10-100 μm, buoyancy and drag can affect particle behavior in electromagnetic sorting (Furlani, 2007) and microvortex cell trapping (Hsu et al., 2008), indicating that microgravity sensitivity should be examined during the design of biodiagnostics.

3. Hardware design

This section focuses on chip design, flow actuation, separation, mixing, and transport to the detection stage for space applications. To operate in the real world, devices must respond to the properties of real samples, so we begin with a discussion of sample properties.

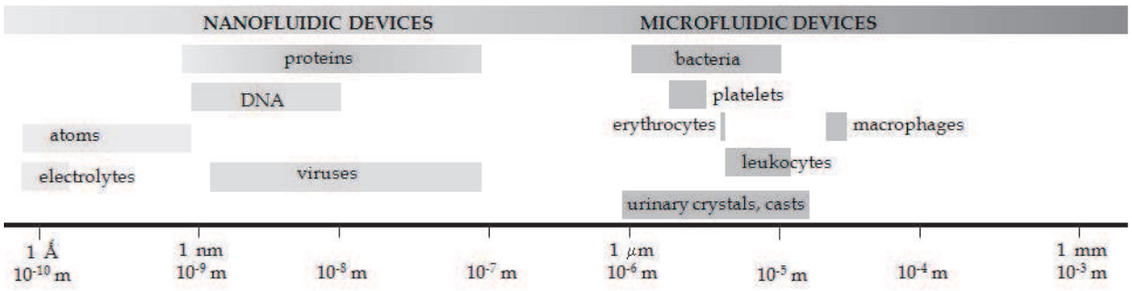

Fig. 1. Length scales of components commonly found in blood and urine samples.

3.1 Sample characteristics

Blood is a mixture of ~55% plasma by volume with ~45% solids content, comprised of red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes, which are the least dense type of cell). The number density of leukocytes is typically 100-800 times less than that of erythrocytes. These cells form the raw material for measurement of hematocrit (volumetric fraction of erythrocytes in blood), counts of erythrocytes, leukocytes, platelets and hemoglobin content. Leukocytes are further distinguished into

	Shape	size (μm)	deform-ability	density (kg/m³)	count (number/μL)	suscepti-bility (SI)
erythrocytes	biconcave, discoid	diameter ~8.5 thickness ~2.3	high	1100	$3.5\text{-}5.5\text{x}10^{6}$	$-9.22\text{x}10^{-6}$ * $-3.9\text{x}10^{-6}$ **
leukocytes	Spherical	diameter ~6-15	low	1070	$3.5\text{-}11\text{x}10^{3}$	$-9.2\text{x}10^{-6}$
platelets	platelike or irregular	1-3	high	lower	$1.5\text{-}4.5\text{x}10^{5}$	

Table 1. Properties of blood cells. Data from (Furlani, 2007) and (Wikipedia, 2011). *deoxygenated, **oxygenated

five basic subtypes. The number density of these white blood cell subtypes vary widely in the blood, as do their size and structure. The least populous subtype, basophils, are found in concentrations of 40-900 cells/μL (Wikipedia, 2011), so that the required sample volume may be larger than a single drop of blood to derive meaningful statistics. More detailed subtyping on rarer white blood cells can provide even more specific diagnostic information, such as the presence of particular cancers or other pathologies. There is much diagnostic value in such information, so significant effort is placed on separating white blood cells and specific subtypes from whole blood. Fortunately, blood cells differ significantly in size, shape, deformability and electrical properties, as shown in Table 1 for healthy adults. As a rheological fluid, the viscosity of whole blood is a function of shear rate and other environmental factors. Its viscoelastic behavior derives primarily from erythrocyte deformability. Blood viscosity is also dependent on its composition and can be correlated to hematocrit and fibrinogen content. Depending on shear rate, the mean whole blood viscosity in healthy people varied from 3.2 to 5.5 mPa·s in one study. With blood cells removed, plasma viscosity decreased to 1.4 mPa·s (Rosenson et al., 1996). As with whole blood, plasma viscosity can be correlated to composition, specifically to fibrinogen, total serum protein and triglycerides (Rosenson et al., 1996). Plasma is primarily composed of water, but also contains essential salts; ionic species such as calcium, sodium, potassium and bicarbonate; and larger molecules such as amino acids, lipids, and hormones and other proteins. Serum is produced from blood plasma by adding anticoagulant, such as ethylenediaminetetraacetic acid (EDTA). Serum is desirable for most assays, with some exceptions such as clotting assays (Hansson et al., 1999).

Platelets are normally platelike in shape, but when activated, they become rounder and long filaments protrude from their surface (Fig. 2(b)). In this state, platelets readily clump together, or aggregate, forming connections via two key proteins, fibrinogen and von Willebrand Factor (vWF). The latter is contained in endothelial cells of blood vessel linings as well as in granules inside platelets. The combination of shear stress and vWF are key players in platelet activation. Shankaran and co-workers identified two stages: one in which the presence of high fluid shear and vWF sensitize the platelet to shear. At later times, platelets can become activated at much lower shear stresses and without the presence of vWF (Shankaran et al., 2003). The process is only weakly dependent on local platelet concentration, indicating that it is fluid shear acting on individual cells, and not cell collisions, that govern the process. Following exposure to a threshold fluid shear stress

τ= 75 dynes/cm^2, they found that a suspension of isolated platelets with viscosity μ=1.1 mPa·s was significantly more likely to become activated (similarly for whole blood at τ=83 dynes/cm^2, μ=3.8 mPa·s). Even a few seconds of exposure can be sufficient to sensitize platelets (Dayananda et al., 2010). The viscosity of the fluid medium is important because it is the shear *stress*, not the shear *rate* reported in many studies, that governs the behavior. (In a homogeneous fluid, shear stress is the shear rate multiplied by viscosity.) Clearly, the microfluidic design must avoid such levels of shear stress. But given the dependence on shear stress history, the sample should avoid wall proximity to limit the potential for adhesion. This is a strong argument for rigorously avoiding fluid separations (recirculating flow cells), which may occur near sharp bends, corners, and steps. It also suggests that sample dilution can be beneficial by lowering fluid viscosity and reducing protein and cell concentration.

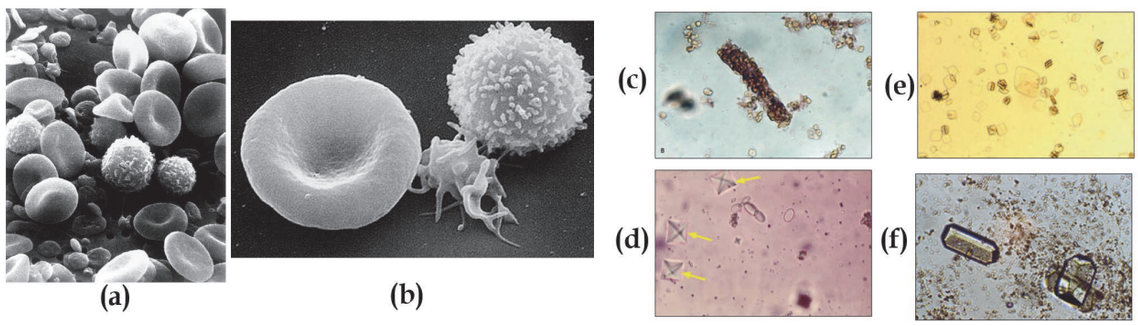

Fig. 2. Solids found in blood and urine samples: (a) blood cells; (b) erythrocyte, activated platelet, and leukocyte; (c) urinary erythrocyte cast; (d) calcium oxalate crystals (arrows) (e) uric acid crystals; (f) triple phosphate crystals with amorphous phosphate.
(a)-(b) reprinted from the National Cancer Institute, Frederick, Md.; (c)-(f) reprinted from the National Institutes of Health Clinical Center Department of Laboratory Medicine, Bethesda, Md.

Urine separation presents a different set of challenges. Although the solids content is far less than blood, the size and other properties of the particulates vary more widely. These can include cells, casts (which include blood and tissue cells that have passed through the renal system, retaining the renal tubule's shape, Fig. 2(c)), proteins, and a wide array of crystals (Fig. 2(d)-(f)). Size, electromagnetic properties, rigidity, and staining propensities are also quite variable. Obtaining well-filtered urine should be straightforward with branching techniques (§3.2.2). Examination of urinary sediment can also yield critical diagnostic information, but it is somewhat more challenging to accommodate in a microfluidic context. Typically, a urine sample of 10-15 mL is centrifuged at ~2000-3000 rpm for five minutes to concentrate the solids content. Pure fluid is withdrawn until ~0.2-0.5 mL remains. A drop of this fluid is then examined under a microscope (Simerville et al., 2005). To examine urinary sediment in the space environment, an effective microfluidic concentrator would be required as well as a means of staining and imaging the concentrated sample.

Saliva is of interest to space biodiagnostics because of its ready availability and marginal invasiveness to the astronaut, particularly since wound healing proceeds more slowly in space (Delp, 2008). Saliva's non-Newtonian viscoelastic behavior is a result of its high mucin

concentration, which makes it a challenging fluid for microfluidic applications. In one study, pre-processing the saliva via filtration through a 0.2 μm membrane was found to remove 92% of total proteins and 97% of the mucins, so that treated saliva could be analyzed in a microfluidic sensor (Helton et al., 2008). However, sensor fouling still remained an issue. Another study used a commercially available sorbent within a microdevice as a preparatory stage for microscale capillary electrophoresis to concentrate hydroxyl radicals while removing undesirable saliva components (Marchiarullo et al., 2008). While these pre-processing stages are not compatible with reusability, they may provide an avenue to pre-processing saliva for use in a reusable device.
Other sample types of interest include examination of potable water for toxin and bacterial content, animal fluids and cell cultures, for which the strategies discussed above are applicable. Throughout the rest of this document, the term "analyte" or "target" refers to the component of the sample that is to be analyzed. An "assay" is simply a test performed on a sample to yield information on the desired target.

3.2 Chip design

For a reusable device, the potential for cross-contamination is a major concern. Designs should therefore minimize residence time of the sample near the walls to minimize the opportunity for adsorption. In general, channels of uniform height without sharp bends, steps, expansions or contractions will have less opportunity to form stagnation zones that could increase sample residence time near the walls. A continuous, uniform flow rate also limits the potential for fluid eddies that could bring the sample into contact with the wall. (On the other hand, devices with unavoidable and persistent stagnation zones could benefit from periodic disruption by pulsatile flow (Corbett et al., 2010.)) Unless a competing design presents substantial advantages and minimal compromises in operation and lifetime, this should be the baseline design of a reusable microfluidic device.
In general, passive processes are preferred for space applications in order to reduce power consumption. The most robust system will have no moving parts, simple geometries, and simple flow controls. Meeting this goal may involve relatively simple design tradeoffs in separation and mixing processes (§3.2.2), but it is more difficult for flow actuation (§3.2.1).
From §3.1, it is clear that for reusable microfluidics, it is advantageous to avoid sample contact with walls. Consequently, droplet-based processing will not be included in this discussion. Sheathing, which surrounds a sample by flowing streams of inert liquid such as buffer, is one means of separating the sample from the chamber walls. This strategy is routinely employed in flow focusing (§3.3). If the sheathing fluid does not mix effectively with the sample, one study showed that the sheathing fluid could even be recycled in an automated fashion (Hashemi et al., 2010). Another microfluidic device encapsulated plugs of aqueous analyte in oil to prohibit sample contact with the biochip surface (Urbanski et al., 2006). Oil and water are examples of two fluids that are immiscible, i.e., they do not mix, but instead maintain a sharp interface.

3.2.1 Flow actuation and control

Fluids handling requires effective means of actuating (initiating) flow, priming, pumping, metering, separating, mixing, and flushing. There are many options for initiating fluid motion in microfluidics. Capillary forces are sufficient to draw blood into a glass micropipette. While this technique could be used to introduce blood into the device, it is

generally insufficient to pump fluid through a microfluidic device (with some notable exceptions (Martinez et al., 2010).) Mechanically, the simplest way to drive flow is through a hydrodynamic pressure difference, produced by connecting the inlet and exit ports to fluid reservoirs of different heights (Shevkoplyas et al., 2005; Simonnet & Groisman, 2006). A 1-m difference in water column height between two reservoirs translates to an overall pressure drop of 10 kPa, which is sufficient to drive flow in an uncomplicated microdevice. This might also be an option on the lunar and Martian surfaces, after taking into account their reduced gravity to roughly 1/6th and 2/3rd earth gravity, respectively.

Pressure-driven flow can also be actuated with syringe pumps. Although they represent an unacceptable penalty on mass, volume and power resources for space diagnostics, they provide reliable, precise control over the flow rate on earth. A manually operated syringe for driving flow is feasible for space operations, but flow control would be more difficult.

Mechanical micropumps include centrifugal, peristaltic, reciprocating and rotary pumps. Displacement pumps apply forces to move boundaries, which in turn move fluid. One example of this class is peristaltic pumping, in which three or more pumping chambers are squeezed in a deliberate sequence with an actuating membrane. Reciprocating pumps initiate flow in a pressure chamber through actuation of a diaphragm. Rotary pumps move fluids by means of rotating, meshing gears. All of these standard techniques for actuating fluid have counterparts at the microscale, but there are also additional options available. Below we describe some of the more intriguing microfluidic flow actuators that could be suitable for space.

Microfluidic networks built on a rotating disk can operate without internal moving parts, using centrifugal force as the sole means of flow actuation (Madou et al., 2006). Many such systems conform to the size of compact discs and can even be used in a conventional CD drive. The current convention is a disposable "lab-on-a-CD" with single-use membranes acting as valves, but this design could conceivably be made reusable with appropriate valving, extraction and flushing functions. Recent innovations with such devices on larger-scale samples (Amasia & Madou, 2010) could make this technique a design choice worth considering for urinary solids concentration.

Bubbles can be used as a type of displacement pump, since they displace liquid during controlled growth. Hydrolysis can be used to generate bubbles with precision to drive flow in a microfluidic channel (Furdui et al., 2003). Deliberate creation of bubbles within a microfluidic device for space, however, should be considered with caution, since bubble management is not a trivial matter.

Other electrically based methods include electrocapillary or electrowetting micropumps, which use an electrical field to dynamically modify the surface charge, thereby controlling the local surface tension. Surface-tension gradients can be generated in a manner that mimics peristaltic pumping. Electrokinetic pumps use electrophoresis and electroosmosis to drive flow. All can be effective in microdevices, since they can be designed to operate at low power and without moving parts. There are a variety of commercial and research-level micropumps and microvalves based on these principles. These techniques represent a viable alternative to micromechanical actuation, but bubble control, surface stability and gravitational independence must be demonstrated over a long lifetime.

Timing, valve control, and well-controlled mixing over a long lifetime are going to be essential features of a successful device. If tight precision on metering, mixing and splitting is needed, one solution with easy computer interfacing may be found using solenoid

actuators that pneumatically control elastomeric valves. One such device reliably manipulated sample volumes down to 5.7 nL (Urbanski et al., 2006).

3.2.2 Microfluidic channel design

The microfluidic network must accept the sample and supporting fluids, perform sample pre-processing such as separation or concentration, provide a means of mixing the sample and reagents or other additives, and transport the fluid to the detection region. For reusable devices, they must also have associated flushing operations performed in them.

Mixing can be achieved passively by introducing texture to walls, placing obstacles in the flow, splitting and recombining fluid streams, or introducing curvature. When two miscible fluid streams are introduced into a single microfluidic channel, the fluids will mix spontaneously via molecular diffusion as they traverse downstream. However, this can require a very long distance because diffusion is a very slow process. Complete mixing over a shorter length can be achieved if additional incentives are introduced, such as convective motion. Convective mixing is added through geometry by channel bending, twisting, and flattening (MacInnes et al., 2007). However, these convoluted flow paths come at the price of increased flow resistance, which imposes increased power requirements (Hsu et al., 2008).

Modification of wall geometry was used to improve immunoassay performance through mixing enhancement (Golden et al., 2007). In this case, antibodies were immobilized at the bottom sensor surface. The target protein was captured from the sample stream as it bound with the antibodies, resulting in a layer of increasingly target-poor solution next to the sensor downstream. By adding grooves at the top of their channel, they promoted mixing over the entire cross-section of the channel. Increased mixing resulted in better delivery of fresh analyte to sensor surface. Other studies have examined in detail the effect of such surface modifications on flow profiles (Howell et al., 2005). Surface patterning can provide effective mixing, but it adds complexity to the fabrication process, and may slightly increase the necessary driving force to move fluid through the system. Most critically for reusable devices, they must be evaluated for fouling potential in the vicinity of the patterning.

Diamond-shaped obstacles force the flow to break up and recombine, providing good mixing at low power over a broad range of flow conditions (Bhagat et al., 2007). The sharp leading edge acts to separate the fluid streams. The design also provides a potential location for a stagnation zone just downstream of the sharp corner at the widest portion of the diamond. In the laminar flows that are typical of microfluidics, such expansions can generate flow separations if the expansion angle exceeds 7° (Panton, 1984). Substituting a slimmer biconvex shape could reduce this proclivity, but it would also reduce the intensity of mixing. The mixing becomes less vigorous by decreasing the span of the obstruction and hence the amount of fluid lateral motion. By eliminating the separated zone next to the obstacle, we have limited the region of increased mixing to strictly downstream of the obstruction. This option increases geometric complexity only slightly, although it introduces new walls into the system. For reusable systems, the fouling potential must be evaluated on the surfaces of the obstruction and weighed against gains in mixing efficiency.

Another technique for passive mixing without tortuosity, splitting, obstructions or surface roughness is through the introduction of curved channels. As fluid rounds the bend, centrifugal forces drive Dean flow, evidenced in secondary flow structures in the form of two counter-rotating vortical structures along the flow direction that span the channel cross-section. Frictional drag on a given particle is proportional to its effective radius in

the flow direction and the net acceleration acting on the particle. Through this mechanism, the mere presence of the curved channel can serve to drive size-based particle separation (Di Carlo, 2009). Any relative motion between the particle and its carrier fluid should also serve to provide additional fluid mixing. This design is attractive for reusability because it enhances mixing without requiring geometric features that introduce fouling potential. In a recent example, a spiral architecture was a core design principle of a reusable blood analyzer prototype for space (Chan et al., 2011).

Electrokinetics-based techniques in mixing (Chang et al., 2007) generally require more attention to lifetime for space applications, since their function is dependent on surface properties and treatments, which can degrade over time (Mukhopadhyay, 2005). Valve functions can be lost due to contamination during usage, although surface geometry can be an aid in this regard (Nashida et al., 2007). Finally, these techniques may not have the same level of control as micromechanical metering (Urbanski et al., 2006).

Careful filtration reduces the likelihood of introducing fouling contaminants to the system, which minimizes clogging and simplifies post-test cleaning. Filtration may be necessary at multiple length scales in sequential stages, based on the constituents in the sample and the assay under consideration. By removing unnecessary components from the sample, it can have the added benefit of improving the signal-to-noise ratio due to nonspecific response in the detection stage. On the other hand, filtration could also remove large molecules, such as some proteins, that may be the target of a particular assay.

Filtration techniques range from brute-force mechanical trapping to elegant biomimetic capture. For filtration in reusable devices, continuously flowing techniques are preferred to mechanical trapping. All separations exploit variations in size, density, deformability, biokinetic and electromagnetic properties among the blood components. In the systems in Fig. 3, blood cells are preferentially directed into specific channels, but are not trapped nor are they subjected to vigorous mechanical forces that could cause cell lysis, or rupture.

Plasmapheresis is the process by which plasma is separated from whole blood. Fig. 3(a) demonstrates the utility of simple bifurcations to extract pure plasma (Yang et al., 2006). Processing time could be reduced by adding pulsatile flow (Devarakonda et al., 2007), but the increased complexity and fouling potential may be of concern for space diagnostics. Another design option is to send the entire fluid stream through a constriction followed by an expansion. In this case, a cell-free layer develops next to the downstream walls, the extent of which is a function of the length and width of the constriction, as well as the flow rate (Faivre et al., 2006). Gentle contraction and expansion flows may serve to further segregate the cells from the walls while providing a plasma-rich region nearer to the wall close to the branch points.

Prototypes for separation often use inorganic analogs for blood cells as a starting point. Although a reasonable analog for leukocytes can be found in appropriately sized and weighted rigid spheres, erythrocytes are neither spherical nor rigid. Studies that carefully design geometries and flow rates to separate out differently sized spherical particles are likely to miss the mark when extrapolating to real-life blood cell separation. Dense suspensions of rigid particles in flowing fluid tend to have a high concentration of the smallest particles immediately adjacent to the boundaries. However, hydrodynamic forces acting on deformable, biconcave erythrocytes drive them to the fastest-moving region of flow, although they are smaller than leukocytes. Consequently, in bifurcating flow (Fig. 3(b)), erythrocytes preferentially choose the higher velocity bifurcation (Yang et al., 2006).

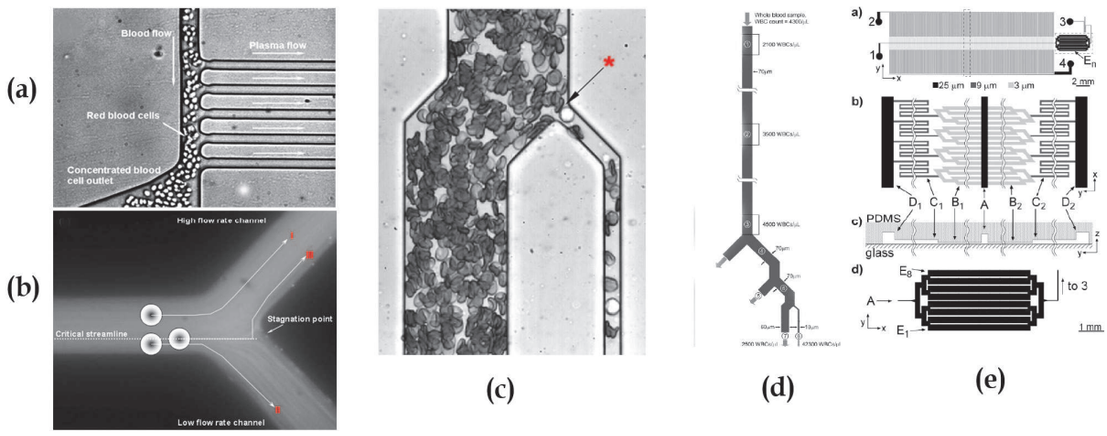

Fig. 3. Continuous flow separation techniques: (a) plasmapheresis through branching channels, (b) erythrocytes exhibit a preference for the faster moving stream, (c) detail of branching technique for leukocyte enrichment from whole blood using the network of (d) leukopheresis geometry for 34x enrichment; (e) leukopheresis geometry for 4000x enrichment. (a)-(b) reproduced with permission from **(Yang et al., 2006)**, copyright 2006, The Royal Society of Chemistry; (c)-(d) reproduced with permission from **(Shevkoplyas et al., 2005)**, copyright 2005, American Chemical Society; (e) reproduced with permission from **(VanDelinder & Groisman, 2007)**, copyright 2007, American Chemical Society.

Blood flow exhibits Poisseuille (parabolic) velocity profiles in microchannels. Erythrocytes favor the faster-moving flow at the center of the channel rather than the slower-moving fluid near the walls. As these cells migrate to the center of the channel, cell/cell collisions tend to drive leukocytes toward the channel walls. The design in Figs. 3(c) and (d) ensured that all of the collisional energy among blood cells was dedicated to driving leukocytes to the walls, providing efficient locations for siphoning off cells (Shevkoplyas et al., 2005). This clever biomimetic technique operated effectively without sample dilution and with simple process control for minimal resource consumption. The lack of dilution and the reliance on collisions may impact fouling potential, however.

Excellent performance in leukocyte separation from whole blood was demonstrated in the device in Fig. 3(e), without fouling over an hour in continuous use (VanDelinder et al., 2007). In a single pass, the ratio of white to red blood cells at the outlet showed a 4000-fold increase. The simpler design shown in Fig. 3(d) showed more modest leukocyte enrichment of 34 times (Shevkoplyas et al., 2005). However, both designs beat "buffy coat" preparation produced by single-spin centrifugation, which provides 10-20 times enrichment. The geometrically complex design of Fig. 3(e) features channels of varying height which intersect at right angles, and requires flow control. Contrast this to Fig. 3(d) which exhibits uniform height, minimal branching at gentle angles, and is driven by a single pressure drop over the entire system. Either design provides adequate enrichment for simple leukocyte differentiation. Space biodiagnostics favors devices that provide adequate function with minimal resources and simple geometries.

Another common method of enrichment uses an array of micropillars strategically placed in the channel (Chang et al., 2005). The physical obstructions preferentially slow down leukocytes without stopping them completely, thus providing enrichment. Many other separation techniques also rely on hydrodynamic principles to separate cells from blood. Electrokinetic, electroosmotic, dielectrophoretic and magnetic forces can also be used for active separation, discussed in depth elsewhere (Lenshof et al., 2010; Salieb-Beugelaar et al., 2010).

3.2.3 Materials choices and fouling considerations

Most prototype biochips are created from materials that are easy and inexpensive to manufacture, particularly polydimethylsiloxane (PDMS). However, such polymers readily absorb small molecules that can interfere with fluorescence measurements (Toepke & Beebe, 2006) as well as nonspecific proteins (Mukhopadhyay, 2005). To mitigate this unfortunate property, surface treatments such as plasma exposure can be used to create a hydrophilic surface. This treatment also improves surface bonding. A great deal of effort is devoted to design of low-fouling surface coatings, but they may not survive in microdevices requiring use over a long lifetime (Balagadde et al., 2005; Mukhopadhyay, 2005). In addition, the non-negligible permeability of polymers to gases imposes the need for good strategies for fluids priming, flushing and bubble control, since liquids within a polymer device will evaporate over time.

More promising materials include silicon and glass, which are less surface-active and less permeable to gases. They maintain integrity over a longer lifetime, and geometric details can be more tightly controlled, especially with silicon. They are, however, more expensive to manufacture (Han et al., 2003). In order to use a glass biochip safely on the Space Station, it must have containment that filters particulates down to 50 μm (International Space Station, 2002). Silicon presents some unusual design possibilities by providing bounding walls that can be dynamically charged to change wetting and adsorption properties. In one design, which also included a low-fouling polymer layer, a controlled electrostatic attraction pulled proteins from solution onto the wall reversibly (Cole et al., 2007). This technique could potentially be used to concentrate urinary protein for detection, filtration, or flushing (with the usual caveats for addressing lifetime and cross-contamination issues).

Regardless of materials choice, it is beneficial to minimize contact between the sample and the wall or sensor to reduce the potential for fouling. To avoid wall interaction entirely, one strategy is to encapsulate the aqueous sample in an immiscible fluid (Urbanski et al., 2006). Aside from fabrication challenges and gas permeability, another concern is the degradation of biochip components, such as electrodes (Chen et al., 2003; Shen & Liu, 2007; Shen et al., 2007; Zhang et al., 2007), which may interfere with detection performance, release contaminants into the device, or affect containment. In some cases, electronics can be placed outside of the channel to prohibit contact between the sample and the sensor (Nikitin et al., 2005). Processes that employ electrical or magnetic fields to drive, mix or separate fluid streams can also be designed to avoid contact of the control elements with the sample.

Materials choices, surface treatments and coatings, the type of reagents and samples can all influence device lifetime and performance. Silicon and glass are good working materials for producing a long-lived, reusable microfluidic device. Polymers are less suitable candidates for space diagnostics due to higher gas permeability, greater potential for fouling, and reduced geometric integrity. Electrodes and other biochip components must not degrade over a lifetime of several years and hundreds to thousands of uses.

3.3 Detection strategies

The primary functions of biodetection are to count particles, detect and/or quantify concentrations of dissolved compounds and visualize particulates. Detection techniques can be based on direct sensing or may require an intermediate step for labeling, binding, or chemical reaction. To minimize reagent usage, reduce sample residence time and fouling potential, reusable design has a strong bias towards detection schemes requiring short incubation periods, fast reaction kinetics, and short detection times.

Since there are many comprehensive reviews cited in §1 and elsewhere that cover the field in great depth, we will focus on optifluorescent detection, which provides great versatility in designing assays using a single detection modality, for both cell counting and massively multiplexed biomarker detection. The popularity of this technique has prompted development of multi-channel laser systems, which increase the multiplexing potential. The capability to differentiate many targets simultaneously is enhanced by recent developments in assay development, discussed in §4.2.

An impressive differentiation of all 5 leukocyte subtypes was recently achieved by Tai and co-workers (Shi et al., 2011). Their approach is based on strategic choice of three fluorescent dyes which stain proteins, nucleic acids, and cytoplasm contents. The resultant fluorescent signatures are sufficient for a two-channel laser detection system to discriminate among all the cell types. This approach was tested on 5μL human blood samples, spiked with purified basophils due to their rarity in whole blood. The differences in the cell's internal structures cause the uptake of the dyes to be proportionally different for each cell type. A scatter plot of red vs. green fluorescence intensity produces 5 distinct regions, which correlate to the 5 cell types. Data points in each cluster are counted to enumerate the number of cells in each category. The resulting measurement agreed well with a commercial assay system in terms of cell subtype percentages as well as overall leukocyte count. In operation, the blood sample will be acquired through a needle integrated with a disposable cartridge, which interfaces with their portable microcytometer. They deliberately designed the system to avoid the need for diluents, which keeps the chip size small. By encapsulating the sample within the chip, the biohazardous waste from sample acquisition and processing is contained and the possibility for cross-contamination minimized or eliminated.

In some cases, visual examination of microstructural detail can add enormous insight into the physical, biological and physiological processes of interest. The International Space Station hosts the Light Microscopy Module, which can perform high-resolution color video microscopy, brightfield, darkfield, phase contrast, differential interference contrast, spectrophotometry, and confocal microscopy. Options include custom-designed laser tweezers for sample manipulation and remote control from earth at NASA Glenn Research Center. Experiments using the device began in March 2011 as this is being written. No results are yet available, but human blood will be one of the early samples examined. These capabilities bring the power of a state-of-the-art terrestrial imaging facility to the Space Station, continuing NASA's shift in focus from ground-based analysis of space-exposed samples to *in situ* analysis in microgravity.

To facilitate ease of use and expand capabilities for bioanalysis, Todd and co-workers are developing an observation platform to interface with the Light Microscopy Module, which adds a substage illuminator and epi-illumination (Todd, 2009). Onboard controllers and actuators can be used to exchange fluids between two small chambers on the platform to initiate a process, fix biological samples or retrieve suspended cells. This device could be used for cell counting and detailed visual examination of cell and plant cultures, animals and human blood, urine, and water samples.

4. Operational design

To function effectively in a space habitat as a general-purpose laboratory, a reusable microfluidics-based biodiagnostic must include strategies for sample acquisition (§4.1), incorporation of new assays (§4.2), and effective flushing/cleaning operations (§4.3).

4.1 Sample acquisition and transport to the microfluidic device

As with any biological diagnostics, protocols for sample acquisition must be established to ensure reliable, repeatable measurements, including skin cleansing to remove potential contaminants as well as efficient, non-contaminating sample acquisition and transport to the microfluidic channels of the biochip. To reduce invasiveness to the astronaut, acquisition of capillary blood is preferred to a venous blood draw. Resource-conscious fingerprick devices for space are under development (Chan, 2009). Biohazardous waste from sharp needles can be reduced through the use of microneedles, which are also better at reducing invasiveness and pain. But they require a means of transporting the sample to the chip in a sterile, bio-contained fashion. For our purposes, the flow driver for sample transport could be integrated into either the lancet/needle side or the device side of the system, depending on which design is most compact or practical. Actuation drivers can be placed on the biodiagnostic device, acquisition device, or both, to supplement capillary forces in bringing the sample onto the biochip. Since there is a discontinuity in the fluid path at the junction of the sample transport device and the chip, acquisition is a key element in device design for bubble-free operation.

Acquisition and transport of a urine sample in space can be a messy procedure. From the standpoint of reusables, the best option would be integration of sample collection with the urine collection system on the spacecraft. Since urine has a much lower solids content than blood, acquisition of a well-filtered fluid sample should be simpler than for blood. Examination of urinary sediment would require a means of concentration. Branching techniques as used for plasmapheresis in §3.2.2 could be considered. More efficient concentration could be achieved with micro- (or milli-) centrifugation (Amasia et al., 2010).

Following sample acquisition, any nondisposable components will require cleaning to return it to a clean state. It may be impractical to clean some components, particularly those at the smallest scale. In this case, the next best goal is to minimize the disposable part of the system.

4.2 Assay design

Assay development is going to be one of the limiting factors in realizing the full capabilities of a massively multiplexed biodiagnostic device. Techniques that require no additional staining, labeling or binding agents are particularly attractive for space use, but a general-purpose system will not be able to avoid the use of additives. For example, opportunities to exploit autofluorescence are only available for a few targets. The biokinetics of some immunoassays are reversible in principle, but performance degrades after a number of binding and unbinding cycles, although gains have been recently made (Choi & Chae, 2009). The least attractive option for space diagnostics is to introduce single-use reagents into the system, but it is unavoidable considering the need for a reasonable range on the assay suite.

The sensitivity and specificity of a given assay will be a function of (bio)chemistry, sensing modality, design and calibration standards, and the fluid matrix in which the target is embedded (Vesper et al., 2005a; Vesper et al., 2005b), as well as the fabrication process. In designing a system that can be used for blood, urine and other sample types, some system efficiencies can be realized through the existence of common assays. For example, measurement of glucose is specified in the crew health requirements for both urine and blood. Moreover, from a medical standpoint, diagnostic value may be improved when both serum and urine data are available, e.g., for osmolality (Pagana & Pagana, 2005).

When reagents are needed, wet chemistry is the most widely used approach for blood analyzers. Stability can be improved by reconstituting dried reagents at runtime (Chen et al., 2005), microencapsulation techniques (Sahney et al., 2006), and stabilizers, particularly in the case of immunoassays (Guire, 1999; Park et al., 2003). Dry chemistry is likely to have the best payoff in the stability of biological recognition elements, such as antibodies. This is the approach taken in urinalysis test strips, e.g., Chemstrips, that have a shelf life of one year after opening the package. Reconstitution of soluble reagents or tethered molecules at runtime is unlikely to present any microgravity-related issues.

Aptamers are bioengineered molecules, usually based on nucleic acids, which may have similar binding affinity to the more conventional antibody. These biorecognition elements may be more stable than antibodies, and have great potential for assay design through the ability to place chemical agents at highly specific binding sites (Cho et al., 2009). Work is progressing rapidly in this area, in part through the support of NASA (Yang, 2008), but these reagents are much less broadly available than antibodies.

Magnetic beads are functionalized by immobilizing antibodies or other biorecognition elements on the bead surface. When exposed to the target, there is a strong affinity for binding. This technique can be used to separate components from the bulk fluid efficiently. These processes can be exquisitely sensitive and are well-suited to sorting rare cell types; and they are discussed comprehensively elsewhere (Furdui & Harrison, 2004; Pamme, 2006). With beads as a reagent carrier, the microfluidic system becomes much more adaptable and resource-conscious. The same microfluidic channels can be re-used, and the set of micron-scale beads introduced at runtime determine which assays are performed. The ability to discriminate among assay signals at the detection stage then becomes the chief bottleneck. At this time, 8-color fluorescence systems have become available commercially, which expands capacity greatly if appropriately fluorescing compounds can be matched to targets. Recent work moves towards expanding the number of fluorescing sensing stations on each magnetic bead, which can also increase capacity (Chan, 2009; Hu et al., 2011). Unfortunately, much of the current work is geared to genomics and proteomics.

Nanostrips are ingenious new reagents that are conceptually similar to the standard urinalysis test strip, but the strip is shrunk a billion-fold down to the micron scale (Chan, 2010). As with urinalysis test strips, each nanostrip can have multiple sensor locations, each of which responds to a different target. The embedded reagents may be antibodies or aptamers tagged to fluorescent molecules that are designed for protein detection, or fluorescent dyes that react with other targets in the sample, such as electrolytes. These small, rectangular nanostrips are similar in size to blood cells, simplifying detection and analysis protocols. A dual-channel laser system measures the fluorescence signatures of both nanostrips and blood cells. For the nanostrips, one channel is dedicated to identifying the strip, so that the system can determine which set of targets is being measured. Essentially, the concentration of dye on each sensor pad creates a bar code for identifying the strip type. The other channel is used for the actual measurement. Quantitative measurements are obtained through analysis of fluorescence intensity at each sensor location. Since the identification channel can easily discriminate many levels of fluorescence intensity to add further differentiation, a set of 5-part nanostrips could theoretically measure thousands of targets from a single sample. At present, nanostrips of up to 7 parts have been fabricated. As with the other systems discussed, a major bottleneck is assay development. Some effort in nanostrip delivery and data analysis techniques will also be needed. But the beauty of this approach is that another limiting factor may become the user's ability to take advantage of nanostrip capacity.

4.3 Flushing and cleaning protocols

The first step in developing flushing protocols is to define what constitutes an adequately clean device. Sterility is a very high standard, especially in an environment like the Space Station without access to an autoclave or common sterilizers, such as bleach or glutaraldehyde. But sterilization between each blood sample is probably unnecessary. High-throughput systems for blood analysis, such as the Shenzhen Mindray Bio-Medical Electronics Co. BC-2800, can run continuously for several days before its tubing and other components must undergo even a routine cleaning with an enzymatic detergent. However, there are few commercial examples of a reusable portable device for guidance in developing cleaning protocols. The simplest strategy for cleaning a microdevice is to flush the system with saline. To assess effectiveness, quantitative measures can be used for comparison, such as fluorescence or color, against pre-test levels (Balagadde et al., 2005) or to some specified reduction, such as 10^{-6} times the reference signal (Verjat et al., 1999).

For a reusable multipurpose analyzer, we must also think in terms of the assays we require the device to handle, the sample components that could be fouling agents, and estimated concentration levels for the sample. The presence of some nonspecifically adsorbed protein X on the wall may not matter much if we are counting cells. We are neither measuring protein X, nor is the attachment or detachment of protein X from the wall likely to interfere significantly with the cell counting. It could compromise the measurement entirely if the target of interest is protein X or if the target is a rare protein Y, which adsorbs to the wall, or displaces protein X, or protein X desorbs independently and has the unfortunate ability to add noise to the measurement of protein Y. In other words, the standards for cleaning the device must be more exacting for measurement of a rare protein than for, say, albumin, which is the most common protein in blood.

Recently, the subject of reusability in biomicrofluidics has received more attention in the literature. Microfluidic devices have begun to report reusability, with applications from the culturing of human lung carcinoma cells with a few re-uses (Jedrych et al., 2010) to the detection of pathogens in livestock with up to 75 assays (Kwon et al., 2010). Self-assembled monolayers can be deposited on the channel wall as capture agents for proteins. Although the binding of proteins to these monolayers is reversible, the degradation of performance with multiple binding/unbinding events has been substantial. Recent efforts in this area have reported that the use of densely packed, short-chain monolayers in conjunction with controlled surface roughness can increase device lifetime to 50 uses (Choi & Chae, 2009). Another fascinating development has been in the area of Surface Acoustic Wave devices, in which an acoustic wave propagates along a solid/liquid interface for detection of binding events. In a device that was designed to produce a wave with a substantial surface-normal component, the force resulting from the surface oscillation was sufficient to remove non-specifically bound proteins from the surface. Also, the steady streaming motion in the fluid driven by the oscillating boundary prevented reattachment (Sankaranarayanan et al., 2010). Another recent work describes the use of nanomechanical resonant sensors in reusable microfluidic channels for the simultaneous detection of interleukin-8 and vascular endothelial growth factor in serum (Waggoner et al., 2010). Continuing efforts in promoting reusability are yielding insights, but much work remains to be done in this area.

Finally, the cleanliness requirements may also vary depending on the end user. Diagnostic data used to treat an individual for a medical condition may require higher standards than biological or biomedical research. All of these areas are ripe for further exploration.

5. Conclusions

In this work, we have explored the principles that can guide the design of a reusable biomedical device for space. The requirements that drive development for space demand far more attention to resource-conscious operation than a similar system designed for earth. However, the efficiencies provided by these considerations can also be of benefit to terrestrial devices by driving down costs and opening up new applications through reduced resource consumption, improved ruggedness, breadth of capability and enhanced adaptability. Some of the essential features for reusability are:

- Continuous flow through the device to minimize sample residence time
- Simple geometries without sharp bends, steps or expansions that could create separation zones or act as bubble traps
- Minimization of sample contact with the wall through low-fouling surfaces and coatings, sample encapsulation, and dilution to reduce sample concentration.

Operation in the extreme environment of space leads to additional design considerations:

- Dry chemistry offers substantial advantages to meet the extended reagent shelf life needed for Exploration class missions
- Bubble control and solids behavior may be different in a reduced gravity environment relative to earth and must be assessed for any spacebound device
- The load on mission resources can be reduced by minimizing the mass, volume, power, and consumables of the system through hardware miniaturization, using shared resources, dynamic reconfiguration capabilities, and the flexibility to accommodate a range of assays on an array of sample types.

Reusable devices are coming closer to maturity for some areas of biological and biomedical research, but there are few examples that are targeted towards a fully integrated blood and urine analyzer for routine medical diagnostics, as well as for a wide range of biomedical research needs. Nevertheless, the basic technology for such a device exists right now. The primary stumbling blocks are integration of sample processing and onboard detection in a single device, the capacity for massive multiplexing and the availability of a broad assay suite. Optifluorescent detection methods are well-suited to reusable design and can accommodate a wide range of assays. Nanostrips can provide massive multiplexing while maintaining a simple, reusable geometry. These approaches hold genuine promise for reaching the much sought-after Holy Grail for portable biodiagnostics: a self-contained, robust, general-purpose assay system for analysis of bodily or environmental fluids.

6. Acknowledgements

The funding for this work was provided by the Human Research Program at NASA Johnson Space Center. Their support is gratefully acknowledged.

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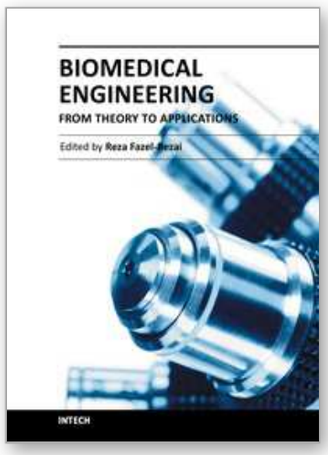

Biomedical Engineering - From Theory to Applications

Edited by Prof. Reza Fazel

ISBN 978-953-307-637-9
Hard cover, 486 pages
Publisher InTech
Published online 29, August, 2011
Published in print edition August, 2011

In all different areas in biomedical engineering, the ultimate objectives in research and education are to improve the quality life, reduce the impact of disease on the everyday life of individuals, and provide an appropriate infrastructure to promote and enhance the interaction of biomedical engineering researchers. This book is prepared in two volumes to introduce a recent advances in different areas of biomedical engineering such as biomaterials, cellular engineering, biomedical devices, nanotechnology, and biomechanics. It is hoped that both of the volumes will bring more awareness about the biomedical engineering field and help in completing or establishing new research areas in biomedical engineering.

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