Considerations for non-invasive in-flight monitoring of astronaut immune status with potential use of MEMS and NEMS devices

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Abstract

The dynamics of how astronauts’ immune systems respond to space flight have been studied extensively, but the complex process has not to date been thoroughly characterized, nor have the underlying principles of what causes the immune system to change in microgravity been fully determined. Statistically significant results regarding overall immunological effects in space have not yet been established due to the relatively limited amount of experimental data available, and are further complicated by the findings not showing systematically reproducible trends. Collecting in vivo data during flight without affecting the system being measured would increase understanding of the immune response process. The aims of this paper are to briefly review the current knowledge regarding how the immune system is altered in space flight; to present a group of candidate biomarkers that could be useful for in-flight monitoring and give an overview of the current methods used to measure these markers; and finally, to further establish the need and usefulness of incorporating real-time analytical techniques for in-flight assessment of astronaut health, emphasizing the potential application of MEMS/NEMS devices.

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Keywords: Cytokines; Immune response to space flight; Astronaut immune system; BioMEMS

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Overview of space flight impact on immune responses

The immune system is a complex network of highly specialized cells and organs that work together to defend the body against foreign invaders. Space flight has been shown to induce varied immune responses, many of them potentially detrimental. Some of these changes occur immediately after arriving in space while others develop throughout the span of the mission (Sonnenfeld, 1998). The causal factors include microgravity, the stress due to high-demand astronaut activities and the social interactions of confinement (Meehan et al., 1993), diet (Heer et al., 1995; Beisel and Talbot, 1985), lack of load bearing (Schmitt et al., 2000; Armstrong et al., 1993), and radiation (Mortazavi et al., 2003). Photobiomodulation, although not frequently mentioned, has been addressed by other researchers (Hug et al., 2001). To date, the only consistent effects on the immune system observed have been a reduction in T-cell counts and a decrease in NK cell concentration and functionality (Sonnenfeld and Shearer, 2002; Levine and Greenleaf, 1998), a reduction in cell-mediated immunity, altered cytokine production (Crucian et al., 2000; Levine and Greenleaf, 1998; Konstantinova and Fuchs, 1991), and constant levels of immunoglobulins (Levine and Greenleaf, 1998). More recent research shows that there is an increased susceptibility to infection under space flight conditions (Mehta et al., 2000, 2004; Aviles et al., 2003). The main concern of a weakening immune system in the closed environment of a spacecraft is the possibility of having altered ability to heal from bacterial and certain fungal, viral, and parasitic invasions. An infection in one astronaut could be difficult to contain from spreading to the rest of the crew under the closed conditions of a spacecraft. Moreover, a weakened immune state could lead to dormant virus reactivation which could have serious implications for a crew trying to return to Earth. When an astronaut experiences these conditions or a combination of them, the body reacts by releasing hormones to counteract the debilitating effects of stress. However, some of these chemicals act as inhibitors of immune system function, and lead to a deteriorated health as the mission progresses. This is of special concern to long-term missions, because it has not yet been determined whether the immune system will reach a new plateau/nominal state.

In space flight, monitoring inflammatory mediators could prove to be useful in determining the presence of infection, as well as aid in following the augmentation or down regulation of immune response, thus, tracking healing. Instances that could precipitate an inflammatory response include: injury (open wound, broken bone, spinal cord injury), trauma, stress, infection, hypoxia (which produces inflammation in several tissues including skeletal muscle: Orth et al., 2005), and hyperoxia (which causes direct injury to epithelial and endothelial cells: Bustani and Kotecha, 2003).

On Earth, diet has a profound effect on the balance and functionality of the immune system; thus, a decrease in the intake of necessary minerals and vitamins will certainly affect the immune system (Beisel and Talbot, 1985). Astronauts essentially follow the recommended daily allowances (RDA) of micronutrients. This was brought on in part by nutrient balance studies performed during the Skylab missions that showed that the absorption of macronutrients and many micronutrients had similar fractional absorptions in space as on Earth (Johnston et al., 1975; Lane and Schoeller, 1999). Other studies have shown that there are major differences in absorption of certain nutrients, as suggested by, for instance, increased levels of urinary excretion of calcium (Smith et al., 1999; Wastney et al., 1999), which are not able to be counteracted by increasing calcium intake because of the increased risk of renal stones (Zerwekh, 2002). Also it has been observed that astronauts are not able to maintain an adequate caloric intake to match their metabolic expenditure of energy (Borchers et al., 2002; Stein et al., 1999), which in turn leads to rapid weight loss followed by accelerated muscle atrophy and bone loss. This becomes a significant consideration when planning a strenuous activity requiring higher energy levels such as extravehicular activity (EVA) (Heer et al., 1995).

Radiation also presents elevated risks for space crews. Hazards exist both in Earth and lunar orbits, as well as on planetary surfaces, and consist primarily of solar energetic particles (SEP), and the protons and highly charged energetic particles (HZE) of galactic cosmic rays (GCR) (Vernikos, 1995). While low-level ionizing radiation can actually stimulate the immune system and has the potential to reduce cancer frequency and increase growth and longevity (Feinendegen, 2005; Kojima et al., 2004; Simko and Mattsson, 2004; Cogoli and Gmünder, 1991), long-term exposure is known to promote the formation of cancer and tumors. Risks increase as humans leave the protective layer of the atmosphere and the Van Allen Belts, a factor that becomes an increasingly crucial problem to interplanetary voyages. Current radiation countermeasures include shielding (including “safe havens” within a spacecraft) and prediction (solar events such as CMEs, SAA and others). Potential also exists for nutritional mitigation of radiation-induced cellular damage (Turner et al., 2002) and pharmaceutical radiation protection, mainly attacking the free radicals that form at the cellular level shortly after exposure (Anzai et al., 2004; Schmitt et al., 1999; Vernikos, 1995; Giambarrresi and Walker, 1989). Other sources of local radiation have been suggested to further influence immune response. Hug et al. (2001) have addressed the possibility of photobiomodulation due to exposure to UV radiation through indoor fluorescent lighting within a spacecraft. Though less studied than the effects of...
Table 1
Space flight stressors that may be associated with altered immune response

- Intense pre-launch training, exhaustion
- Launch g-loads, vibration
- Microgravity
- Isolation
- Radiation
- Lack of load bearing
- Microbial contamination; increase in atmospheric pollutants (recycled air and water)
- Changes in barometric pressure (pre and post EVA protocols)
- Limited personal hygiene
- Sleep disruption and changes in circadian rhythm
- Space Motion Sickness (SMS)
- Unbalanced nutrition
- Psychological dynamics of confined environments
- Pressure of high-performance expectations

Solar radiation, the authors indicate of the possibility of cumulative effects on the depression of NK cells by persistence of cis-urocanic acid (the photoinduced product of its isomer, trans-urocanic acid), known to cause immune suppression on skin, if astronauts have been heavily exposed to direct sunlight due to outdoor activities pre-flight. Radiation continues to be one of the most serious, impending health threats for long-term space travelers.

Table 1 summarizes the primary stressors that are associated with alterations of the immune system response during space flight.

Immunological research using human test subjects

In-flight studies

Studies of human physiology in space can be traced back to the early 1960s when Russian cosmonaut Yuri Gagarin became the first man to enter low earth orbit (LEO). To date, immune response studies have mainly been performed in vitro and ex vivo (rather than in vivo) during space flight and most have focused on cell-mediated immunity (Sonnenfeld and Shearer, 2002). Some authors propose that adaptation of the immune system occurs in stages of subsequent weakened responses (Cogoli, 1981). Others have indirectly supported this hypothesis by saying that serum protein fluctuations point to an adaptation mechanism as well (Criswell-Hudak, 1991). Space flight studies are filled with conflicting data, perhaps because differences in experimental conditions and mission duration have affected immune responses in undetermined ways. A few researchers have suggested that major discrepancies in findings are due to methodological differences (Borchers et al., 2002).

One of the trends observed in space flight research has been unaltered concentration of antibodies. In the Apollo missions, immunoglobulins were observed to remain constant except for IgA which was measured to significantly increase in about 50% of astronauts on the recovery day after landing (Kimzey et al., 1975 as cited by Criswell-Hudak, 1991). Pre-flight, in-flight, and post-flight serum samples were analyzed for immunoglobulin content on an early Shuttle flight with duration of 10 days (Voss, 1984). Results showed minor fluctuations in IgG, IgM, IgA, IgD and IgE concentrations, concluding that microgravity’s effect on immunoglobulins was insignificant. Other substances such as circulating catecholamines, epinephrine and norepinephrine, have been observed to remain constant during flight (Kvetnansky et al., 1988) but levels have been found to increase significantly after landing (Pierson et al., 2005; Meehan et al., 1993).

On the other hand, cell-mediated immunity incurs noticeable changes in space. Among these, a reduction in T-cell counts (Sonnenfeld and Shearer, 2002), altered leukocyte distribution (Stowe et al., 1999; Meehan et al., 1993), leukocyte blastogenesis (Cogoli, 1993 as cited by Sonnenfeld, 2002), a decrease in NK cell concentration and functionality (Levine and Greenleaf, 1998), and depression in delayed-type hypersensitivity (DTH) responses (Taylor, 1993; Gmünder et al., 1994 as cited by Sonnenfeld, 1998) have been reported. In a study conducted on 25 astronauts and 9 control subjects (samples obtained pre and post flight for four Shuttle missions ranging from 5 to 11 days in duration), Kaur et al. (2004) found that the number of neutrophils increased an average of 85% from pre-flight baseline levels. Phagocytic capacity and oxidative burst capacity were also found to be significantly lower than controls for flights over 9 days ($p=0.003$ and $p<0.001$, respectively). No consistent changes in degranulation or expression of surface markers were able to be determined. No substantial deviations from normal immunological parameters were observed in Russian cosmonauts after 197 days of space flight except for decreased cytotoxicity after returning to earth (Konstantinova et al., 1995).

To study DTH responses, skin tests were performed during flight and found to be inhibited when compared to ground test subjects during both short and long-term flights (Taylor, 1993; Gmünder et al., 1994 as cited by Sonnenfeld, 1998).

Latent virus reactivation has been shown to occur in short-term missions and is of concern for long-term space flight. Mehta et al. (2004) gathered saliva samples from eight (8) astronauts before, during and after flight and found that the Varicella Zoster virus (VZV) was present in 30% of all the 200 samples taken during and after flight. No VZV activation was found on any of the ground controls. The authors concluded that VZV can activate sub-clinically in healthy individuals after non-surgical stress. In another study conducted by Pierson et al. (2005), 16% of in-flight samples from 25 astronauts showed Epstein–Barr virus (EBV) DNA presence, which was significantly greater than pre or post-flight ($p<0.05$) values. EBV activation has also been observed after space flight (Stowe et al., 2001a). In a study conducted on 28 astronauts, 11 showed post-flight EBV activation, along with increased urinary cortisol by 100% and catecholamines by 200% compared to astronauts with no EBV reactivation. Shedding of cytomegalovirus (CMV) in urine and increased CMV antibody concentration were also measured in-flight for the crew of STS-95 (Stowe et al., 2001b).

Cytokine levels have been shown to be significantly affected by space flight conditions. Cytokines are chemical messengers that directly relate to immune response and can be associated to a range of diseases. They are classified as interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), lymphokines, chemokines, growth factors, colony stimulating factors (CSF) and stress proteins (Stvrtinova et al., 1995). In studies performed...
on astronauts by measuring urinary levels of several cytokines and cortisol, an increase in urinary IL-6 excretion was observed on the first day of space flight and two subjects had markedly increased IL-6 excretion rates after landing (Stein and Schluter, 1994). In another study in which whole blood samples were collected from 27 astronauts before and after flight, Crucian et al. (2000) observed IL-2 production to decrease after space flight for three different T-cell subsets (CD3+, CD4+ and CD8+) as well as a decreased IFN-gamma production in the CD4+ subset. They concluded that these alterations could either be a result of microgravity or readaptation to 1 g.

A variety of other hormonal changes have been observed before, during and after flight in both astronauts and cosmonauts. Cortisol levels are associated with physiological and psychological stress. This glucocorticoid released by the adrenal glands is known to affect metabolism and to have both anti-inflammatory and immunosuppressive effects. Because of this, one would expect cortisol levels to increase on launch and landing days, due to added levels of stress from these events. Stein and Schluter (1994) observed increased cortisol concentration on the first day of space flight. Kaur et al. (2004), however, recorded higher values for urinary cortisol 10 days pre-flight compared to samples examined immediately after landing (3 h) and in subsequent days (3 days). Other researchers have suggested that there is a mission duration dependence governing neuroimmune responses after space flight. Stow et al. (2003) compared cortisol and urinary catecholamine values for a 9-day and a 16-day mission. They found that cortisol levels were significantly lower for the shorter mission while catecholamines were significantly higher for the same 9-day flight. They suggested that these results point to sympathetic nervous system response dominance for shorter flights, while longer flights are affected primarily by glucocorticoids and other cumulative microgravity effects.

These results indicate that immune response is altered by parameters that might not normally be considered significant, such as, in this case, mission duration. It is, therefore, reasonable to postulate that to be able to fully understand the effects of space flight stressors on the body and immune system, additional insight is needed.

**Ground analogs to space flight**

The challenges encountered during space flight have been compared to those faced in similarly stressful operational conditions such as in submarines, at polar stations and on oil platforms (Schmitt and Schaffar, 1993). In space flight, immune suppression has been associated with various physiological and psychological stressors encountered during launch and landing, microgravity exposure, overtraining, malnutrition, Space Motion Sickness (SMS), as well as factors associated with living in the confined environment of a spacecraft, including the risk of increasing atmospheric pollutants that tend to accumulate with prolonged human activity in a closed system. Deep sea diving is another activity that can be considered as a unique space flight analog in light of two main common stress conditions. First, in case of emergency, immediate return to Earth’s surface is not always feasible; and second, the astronauts, just like deep sea divers, experience numerous variations in atmospheric pressure (Schmitt and Schaffar, 1993). In deep sea diving, neutrophil granulocyte activity has been shown to increase (Benestad et al., 1990) which, in turn, results in a decreased resistance to skin and other infections. On that note, Schmitt and Schaffar (1993) commented that during early space flights, astronauts frequently suffered from otitis and skin infections, suggesting the connection to the conditions experienced by deep sea divers.

Gleeson (2002) conducted a study on relevant markers of overtraining in athletes. Athletes are exposed to similar stresses that can impact the immune system as those of astronauts; including vigorous exercise routines, sleep deprivation and malnutrition, and the psychological expectations for sustained high performance. During prolonged periods of intense training, athletes are observed to have negative mood swings, become fatigued, and in many instances, reported to be more prone to getting sick. Other commonly reported physiological and psychological responses include underperformance, sleep disturbance, loss of appetite, and an increased early morning/sleeping heart rate. Gleeson’s study explores possible immunological and biochemical markers that point to impending overtraining, and not surprisingly, some of these concur with those found in studies performed on astronauts (Sonnenfeld, 1999, Stowe et al., 2001a). Gleeson concluded his study by proposing a battery of tests, both physiological (blood chemistry, heart rate measurements) and psychological (mood tests) as a method for monitoring athletes at risk of overtraining.

Antarctica has been used as different kind of space flight analog. The inevitable isolation, inhospitable winter conditions and limited accessibility to the rest of the world make it suitable to simulate some of the more challenging social aspects of space flight. Antarctic research has encompassed a wide variety of stressors ranging from the psychological dynamics of confinement caused by long periods of isolation (Palinkas et al., 2000) to cold physiology, UV light effects, chronobiology, epidemiology and the physiological responses induced by the stress of living in the hostile environment, including immune system alterations (Olson, 2002). Antarctic studies have shown that immune suppression and inflammatory responses, including dormant virus activation, occur in crewmembers. Table 2 summarizes these findings relative to responses observed in space flight. Seasonal immune variations have also been indicated (Francis et al., 2002; Shearer et al., 2002), but the causes are not clear.

Head-down tilt bed rest (HTBR) studies (6°) are used to mimic certain aspects of space flight such as fluid shift, muscle atrophy, decalcification and cardiovascular deconditioning, with severity varying with the length of the experiments. Immunological studies conducted using HTBR, however, have shown that this method does not entirely reproduce the changes in immune responses observed after space flight (Schmitt et al., 2000). Results vary from one HTBR study to another, and the immunological patterns observed lack consistency when cross-
examined with previous in-flight results (see Borchers et al., 2002 for a review of these). Space flight obviously introduces other factors that influence the human body in ways that ground studies cannot replicate.

It should be noted that, if microgravity (and therefore, lack of load bearing) and radiation are eliminated from the list of stressors presented in Table 1, the remaining stressors are common to these aforementioned space flight analogs on the ground.

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antarctic research</th>
<th>Space flight</th>
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<tr>
<td><strong>Cell-mediated responses:</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>T-cell and B-cell functionality and concentration</strong></td>
<td>Decreased significantly. Significant elevated incidence of anergy (diminished reactivity to antigens). Lowered tetanus antibody levels. (Thom and Lugg, 2002; Muller et al., 1995) Diminished cell-mediated immunity on more than half of the subjects (Mehta et al., 2000)</td>
<td>Reduction in T-cell counts. Decrease in NK cell concentration and functionality (Sonnenfeld and Shearer, 2002; Levine and Greenleaf, 1998) Number of neutrophils increased an average of 85% from pre-flight levels. Phagocytic capacity and oxidative burst capacity significantly lower than controls ($p&lt;0.003$ and $p&lt;0.001$, respectively). Degranulation remained constant (Kaur et al., 2004) Decreased cytotoxicity observed after returning to earth (Konstantinova et al., 1995)</td>
</tr>
<tr>
<td>Depression of cutaneous delayed-type hypersensitivity (Tingate et al., 1997)</td>
<td>No significant changes in T and B lymphocyte subsets (Muller et al., 1995) 50% reduction in T-cell proliferation to mitogen PHA (Lugg and Shepanek, 1999) Th1-skewed immunity (Th1/Th2 ratio increased significantly). Valpha24Vbeta11 NK cells significantly increased midpoint isolation (Shirai et al., 2003)</td>
<td></td>
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<tr>
<td><strong>Humoral immunity</strong></td>
<td>No significant changes in immunoglobulins (Muller et al., 1995)</td>
<td>Constant levels of immunoglobulins (Levine and Greenleaf, 1998) All immunoglobulins observed to remain constant except for increase in IgA after landing (Kimzey et al., 1975)</td>
</tr>
<tr>
<td>Normal antibody response to bacteriophage phi X-174 (Shearer et al., 2001) No IgG changes during expedition. Unexplained lower salivary IgA and IgM levels during first 4 months of the year (Francis et al., 2002)</td>
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<td><strong>Cytokine concentration</strong></td>
<td>Changes in production of IL-1, IL-2, IL-6, IL-1ra and IL-10 (Tingate et al., 1997)</td>
<td>Increased urinary IL-6 excretion on the 1st day of space flight; two subjects had markedly increased IL-6 excretion rates after landing (Stein and Schluter, 1994). IL-2 decrease after space flight; not before (Crucian et al., 2000)</td>
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<td>Dramatic decrease in serum cytokines throughout isolation (TNF-alpha, IL-1ra IL-6 and IL-1beta) (Shirai et al., 2003) Time-dependent increase in IFN-gamma and decreases in IL-10 and IL-1ra at individual time points during isolation (Shearer et al., 2002)</td>
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<tr>
<td><strong>Virus shedding and reactivation</strong></td>
<td>Increase in latent virus shedding (Shearer et al., 2002) Increased reactivation and shedding of EBV in all subjects in at least one instance (Mehta et al., 2000)</td>
<td>Shedding of CMV and increased concentration of CMV antibodies in-flight (Stowe et al., 2001b) 16% of in-flight samples from 25 astronauts contained EBV DNA. Significantly greater than pre- and post-flight ($p&lt;0.05$) (Pierson et al., 2005).</td>
</tr>
<tr>
<td>Increase in plasma epinephrine and serum TSH (Xu et al., 2003)</td>
<td></td>
<td>Constant epinephrine and norepinephrine during flight (Kvetnansky et al., 1988). Increased after flight (Meehan et al., 1993). No significant changes in urinary levels 3 h and 3 days after landing compared to 10 days pre-flight (Kaur et al., 2004). Significant increase ($p&lt;0.05$) of epinephrine and norepinephrine than pre-flight values (Pierson et al., 2005). No significant changes in urinary levels 3 h and 3 days after landing compared to 10 days pre-flight (Kaur et al., 2004). Increased cortisol levels on 1st day of space flight (Stein and Schluter, 1994)</td>
</tr>
<tr>
<td><strong>Catecholamines</strong></td>
<td>Increase in plasma epinephrine and serum TSH (Xu et al., 2003)</td>
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<tr>
<td><strong>Cortisol</strong></td>
<td>Low cortisol concentration pre-departure (Muller et al., 1995)</td>
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<td>Significant increase and decrease seen in various subjects when circadian rhythms were free-running (Kennaway and Van Dorp, 1991) Higher cortisol concentrations observed during Austral winter than March values (Sawhney et al., 1995)</td>
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EBV: Epstein–Barr virus, CMV: cytomegalovirus, TSH: thyroid-stimulating hormone.

**Immune responses to space flight in animal and cell culture research**

Animal in-flight immunological studies have mostly been confined to the use of rats and rhesus monkeys, while ground analogs have been extended to include the use of mice (Sonnenfeld, 2003). Mice offer the conveniences of lower cost and less space required for experimental studies,
their whole genome has been sequenced and they carry roughly 90% of the genes responsible for inheritable human diseases (Simske et al., 2003). Rats have mainly been used due to space housing constraints, even when they are not the most suitable animals for immunological studies (Sonnenfeld, 1998).

Most of the in-flight animal research has been carried out on board the US Space Shuttle and Russian COSMOS biosatellites. Some findings of studies performed with rodents include loss of resistance to infection (Sonnenfeld et al., 1988), inhibited NK cell activity (Rykova et al., 1992), reduced capability for wound healing (Davidson et al., 1999), inhibited INF-gamma while IL-3 production remained unchanged (Gould et al., 1987) and a reduction in lymphoid organ size (Congdon et al., 1996). A study performed on pregnant rats showed that while the parents’ immune responses were affected by the conditions of space flight, their offspring showed no alteration to normal ground values (Sonnenfeld et al., 1998). On a study conducted using rhesus monkeys, Sonnenfeld et al. (1996) found inhibited IL-1 production and decreased response to colony stimulating factor (CSF) on bone marrow cells.

Ground research has enabled the simulation of some isolated aspects of space flight such as lack of load bearing, cephalic fluid shift, and loss of bone and muscle mass, mostly through rodent models such as hindlimb or tail suspension. Antiorthostatic suspension experiments on rats have shown a reduction in thymus size, similar to that reported in flight experiments (Steffen and Musacchia, 1986). Other studies have observed constant levels of immunoglobulins (Sonnenfeld et al., 1998), unchanged neutrophil activity (Miller et al., 1994), and inhibited interferon alpha/beta production (Sonnenfeld et al., 1998). Susceptibility to infection has also been shown. In a study conducted by Aviles et al. (2003), antiorthostatically suspended and control mice were subcutaneously injected with Pseudomonas aeruginosa and compared for mortality, bacterial loading and corticosterone levels. Results showed that mice under antiorthostatic suspension had increased levels of corticosterone, a reduced ability to clear bacteria from organs and a significant rate of mortality over those in the control group. P. aeruginosa bacteria are known to cause pulmonary diseases, which present a serious threat to humans in long-term space travel. A urinary tract infection with these bacteria was encountered in one astronaut during the Apollo 13 mission (Aviles et al., 2003).

Animals are also used in the development and study of countermeasures to immune suppression. Chapes et al. (1999) explored the possibility of reducing immunological flight-induced effects by using insulin-like growth factor-1 (IGF-1) on rats. They found that flight animals had significantly higher corticosterone levels, enhanced cytokine levels and NO secretion by peritoneal macrophages, as well as reduced agonist-induced lymph node cell proliferation. Ground controls treated with IGF-1 had a significant increase in spleen weights, but the flight group did not. The authors concluded that while IGF-1 can ameliorate some of the effects of space flight, its normal functionality can be altered by space flight itself. Another pharmacological countermeasure has been studied by Aviles et al. (2004). The authors have shown that the use of active hexose correlated compound (AHCC) enhances immune response in mice as shown by increased cytokine production with predominant Th1 cell response and restored peritoneal cell functionality.

Finally, cell cultures have been employed for various immunological analyses through the use of clinostats, bioreactors, and centrifuges. These analog systems help to gather a basic understanding of behavioral trends of cells in space; however, they cannot completely simulate the complexity of the interactions within the dynamic systems of a human or animal, and are not discussed in depth here. Cogoli (1996), Hashemi et al. (1999), Bakos et al. (2001), Savary et al. (2001) and Borchers et al. (2002) provide reviews of immune related cell culture studies relevant to space flight.

Need for in-flight measuring capability

Because of its dynamic nature, coupled with the lack of adequate in-flight analytical capabilities, it has been especially difficult to draw firm conclusions about the behavior of the immune system from in-flight research. Many studies have focused on the chemistry of blood before and after space missions; however, an improved understanding of how the immune system is affected may be better achieved if biological systems are analyzed while the conditions of microgravity exist. While numerous studies have been performed on rodents (Sonnenfeld et al., 1990; Armstrong et al., 1993), there are inherent uncertainties with the extrapolation of rodent data to humans, and many of the ground studies performed using both human subjects and animals have not always correlated with their in-flight counterparts (Borchers et al., 2002; Sonnenfeld, 2003). Immune suppression in astronauts has been reported (Levine and Greenleaf, 1998), but the methodology of supporting work has not been consistent enough to elucidate the mechanisms that promote such behavior (Borchers et al., 2002). Therefore, more human testing is required to understand the potential immune system changes due to long-term habitation under microgravity conditions.

Current monitoring challenges

Sampling in space and real-time analysis

Obtaining biological samples in microgravity is not a straightforward task. Not only does drawing blood or collecting other human fluids such as urine prove to be a cumbersome task, but also sample preservation by freezing in environments such as the International Space Station (ISS) is not currently viable due to the lack of a suitable refrigerating apparatus on board. Furthermore, even if sample preservation was available, the immune system’s pool of cells and interrelated chemicals do not generally remain constant with time. Samples may degrade or continue to react to the different array of stimuli of space flight, no longer providing accurate information about what occurred at a particular mission time frame. Real-time analysis is therefore necessary to understand what is happening to the body at a specific moment during flight.
Blood is usually the fluid of choice when it comes to physiological assessment, because of the vast amount of information that can be gathered from it. Continuous sampling, however, is not only cumbersome, but also produces a physiological stress that could alter immune response itself. Thus, it is desirable to find alternate non- or minimally invasive ways to gather samples. Oral fluids have been used as one alternative to serum analysis. Some applications have been for the detection of the HIV virus (Nishanian et al., 1998) and trace drugs such as cocaine and amphetamines (Samyn and van Haeren, 2000). Urine has been used to measure many parameters relevant to space flight, including cytokine concentration (Stein and Schluter, 1994). However, no formal set of criteria or standard has been established on how to relate concentration of immune markers in fluids like saliva, urine and sweat to serum concentrations. Other fluids such as breath have been proposed to monitor inflammatory responses (Kharitonov and Barnes, 2002) and are discussed further in the following section.

Many molecular markers exist in body fluids that can give an indication of immune system response. The next logical step after identifying those substances that are indicative of specific immune system response is to develop a method to quantify them in a real-time fashion.

**Candidate immune function markers**

An immunemarker is a substance (organic or inorganic) that plays a role in a specific metabolic process such that it can be traced and correlated to a particular immune function. The need for real-time, non-invasive sampling and analysis in space is evident; however, what remains under debate is what substance (or set of substances) would provide a good assessment of in vivo overall state of the immune function. Selection is complicated, as certain biochemicals have multiple functions across body systems such as the immune, endocrine and respiratory systems. A good marker should present characteristics that uniquely distinguish it from other chemical species and should have low cross-reactivity. Unfortunately, assessing the immune system has proven to be complex in the sense that markers typically have to be evaluated in groups rather than by individual behavior.

Many compounds can serve as indicators of inflammatory responses. Antibodies in saliva have been proposed as markers of infection, one of them being salivary IgA. Salivary IgA is considered to be an important “first line of defense” mechanism within the oral cavity against upper respiratory diseases and has been used as a marker to diagnose and predict risk of infection in athletes (Gleeson et al., 1999). In a study conducted on elite swimmers over a 7-month period, Gleeson et al. (1999) was able to significantly predict ($p = 0.05$) the number of infections based the IgA level decrease trend after increased periods of exercise. Using saliva to analyze for IgG, Loeb et al. (1997) attempted to achieve non-invasive detection of infection with *Helicobacter pylori*. The study, however, failed to successfully detect the presence of the bacteria. In a previously mentioned study also conducted by Gleeson (2002), salivary cortisol, urinary steroids, catecholamines, T-cell ratios, and cytokines were proposed as useful indicators of overtraining in athletes. Espinosa and Bermudez-Rattoni (2001) have used IL-2, IL-6, and TNF-alpha as markers of psychological stress while Hassig et al. (1996) suggested correlating IL-6 levels along with skin tests against infectious diseases to stress immune activation.

Kharitonov and Barnes (2002) suggest that analysis of various biomarkers in exhaled breath including, for instance, volatile organic compounds (VOCs), allows completely non-invasive monitoring of inflammation and oxidative stress in the respiratory tract for diseases like asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and interstitial lung diseases. They advocate for the simplicity, repeatability and non-invasiveness of the analyses, and anticipate rapid development of even faster biosensors in the future. In space flight, the value of the biomarkers in exhaled breath could be applicable to other states of health when combined with additional physiological information.

Other suggested markers involve cellular rather than biochemical compounds. The distribution of T-cell subpopulations has been shown to be an important indicator of immune function (Sonnenfeld, 2002). The CD4+/CD8+ ratio has been used as an indicator of immune suppression in patients with AIDS and observed to generally increase after space flight (Borchers et al., 2002).

Sonnenfeld (2002) comments on the findings of Lyte and Ernst (1993) involving levels of catecholamines such as norepinephrine. Lyte and Ernst (1993) demonstrated that the presence of norepinephrine can enhance in vitro growth of gram-negative bacteria and increase the production of virulence-associated factors. These two findings point to the potential health risk that could arise if immune response is overridden by viral and bacterial infections in space. While some flight studies have found constant epinephrine and norepinephrine levels during flight (Kvetanský et al., 1988) and no significant changes in urinary levels of catecholamines after landing compared to pre-flight values (Kaur et al., 2004), others have observed a significant increase post-flight (Meehan et al., 1993; Pierson et al., 2005).

As discussed in the preceding sections, cytokines have been previously used as markers of immune competence in space research. These biochemicals are of low molecular weight and simpler structure when compared to antibodies or hormones. Inflammation and bone resorption are processes usually triggered by cytokines. The pro-inflammatory cytokines IL-1, IL-6 and TNF-alpha have been related to osteoclast resorption stimulation (Riggs, 2000). For example, IL-1 beta and TNF-alpha are able to act locally to induce bone and cartilage resorption, and may stimulate the production of other enzymes or hormones to amplify the destructive processes in the joints (Ou et al., 2002).

Whether or not measuring cytokines alone offers a sufficient assessment of immune response, it certainly provides a large amount of information that can be extrapolated to patterns of immune functionality. Cytokine responses are both systemic and localized. This has to be taken into account to determine the...
IL-6 Plasma (1.8±0.5 pg/mL,男: 3.0±0.6 pg/mL, 女: 1.5±0.5 pg/mL)

TNF-α Serum (0.75±0.05 pg/mL, 女: 0.65±0.05 pg/mL, 男: 0.85±0.05 pg/mL)

ACTH Serum (0–78.1 pg/mL)

Epinephrine Plasma (10–200 pg/mL)

Norepinephrine Plasma (80–520 pg/mL)

Cortisol Plasma (80–520 pg/mL)

Overall state of health of a patient based on sampling. By studying cytokine responses, it might be possible to gather additional information about the interactive patterns of various systems within the body, for example, the immune system and the skeletal system, which is important in understanding the relationship between immune suppression and bone loss in space.

Monitoring of both cytokines and hormones has been used to detect clinical illness and assess its severity (Barnes, 2001; Gonzalez et al., 2003; Papadakos and Haisma, 2004; Torre-Amione et al., 1996). Emphasis in the literature is on the possible predictive value of IL-1, IL-2, IL-6, and TNF-alpha, as well as other substances such as cortisol, ACTH, catecholamines (epinephrine, norepinephrine) and their respective roles in sickness mechanisms. Table 3 is a select summary of relevant biomarkers and their correlation to disease.

**Review of relevant ground-based studies utilizing cytokine monitoring**

Many studies have been performed using cytokines to monitor stress-related immune suppression. Suzuki et al. (2000) studied cytokine levels on athletes before and after they ran a 42-km race. The researchers found that cytokines such as IL-4 and TGF-beta remained unchanged while others such as IL-12 and TNF-alpha could not even be detected by the most sensitive bioassays. The most significant result obtained from their study was the rise in concentration of the inhibitory cytokine IL-10, the hormone cortisol (known to cause immune suppression), IL-1 and IL-6, the latter increasing by more than 100 times. In contrast, IL-2 concentration decreased by 32%, which the authors attributed to nitrogen oxide production. Mackinnon (1998, as cited by Gleeson, 2002) refers to increases in the plasma concentration of inflammatory cytokines such as IL-1, IL-2 and IL-6, as other indicators of overtraining.

Cytokines such as IL-1, IL-2, IL-6, IFN-gamma and TNF-alpha are produced by both the immune and neuroendocrine systems and have been found to produce intense effects on the hypothalamic pituitary axes (Ferenicik and Stvrtinova, 1997). Since neuroendocrine processes that modulate immune function include mental and physical stress, changes in the neuroendocrine system could lead the organism to have reduced resistance to infection and malignancies.

Fisman et al. (2003) propose in their atherosclerosis and type 2 diabetes studies that cytokines share a common effect on both...
diseases and that these could be classified as “good, bad or aloof”. In their study, for example, they categorize IL-1, IL-2, and IL-6 as “bad” (among others), IL-4 and IL-10 as “good”, and IL-5 and IL-9 as “aloof”. The importance of their observation lies in relating one disease to the development of the other, thus increasing potential for clinical forecasting based on cytokine levels, and also to be able to control the pharmacological effects of these cytokines on multiple systems in the body.

A low type-1 (Th1) to type-2 (Th2) cytokine ratio has been related to suppressed cell-mediated immunity and allergic reactions (Suzuki et al., 2002). In a study conducted on students without a history of asthma, results showed that levels of Th1 cytokines decreased (IFN-gamma and IL-2) while levels of Th2 cytokines increased (IL-4 and IL-6) during exam periods, relative to normal class time baselines (Kang and Fox, 2001). This indicates that, perhaps, an up-regulation and down-regulation of these cytokines could be expected as a response to a stressful situation (Th2 cytokines are also associated with the worsening of asthma). Hassig et al. (1996) suggest that the dampening of cortisol and the hypothalamic hormone dehydroepiandrosterone may lead to reduction of stress-induced immune suppression caused by a low Th1/Th2 ratio.

IL-6 is a pleiotropic cytokine that influences many biological processes and is produced by various cell types including activated monocytes, B and T lymphocytes, fibroblasts and endothelial cells (Gonzalez et al., 2003). It plays many roles including B-cell and cytotoxic T-cell differentiation, proliferation of T-cells and endothelial cells, and stimulation of the production of acute phase proteins by hepatocytes (the chief functional cells of the liver). It can also act as an anti-inflammatory substance (La Flamme and Pearce, 1999). In this respect, IL-6 has been found to impair antibody production when its production is induced in wildtype (WT) mice (La Flamme and Pearce, 1999). It has been shown that, besides taking part in immune functions, IL-6 enhances bone resorption by increasing osteoclast activation (Bornfauk et al., 1997). This may indicate that IL-6 assessment could serve a dual purpose for monitoring both the immune system and bone resorption processes, which would be useful for treating conditions such as osteoporosis as well as loss of bone in space. Klein et al. (1989 as cited by Bornfauk et al., 1997), also suggest that IL-6 may be involved in tumor growth.

**Current techniques to measure cytokines**

Traditional cytokine sensing methods are based on the use of enzymes and antibodies as receptor sites in techniques such as fluorescence-based immunoassays. The enzyme-linked immunoassay (ELISA), also known as “sandwich assay”, is the most frequently used method for detecting and quantifying cytokines. Other fluorescence methods include radioimmunoassay and flow cytometry derivatives such as multiplex assays that can assay 100 cytokines at a time within one sample with sensitivities ranging from 0.13 to 2.81 pg/mL (R&D Systems, 2005). Among the few and most recent techniques of analysis that allow quick fluorescent monitoring of certain cytokines (3–10 min) is the evanescent wave sensor, which utilizes fiber optics to provide real-time kinetic measurements in the sensitivity range of an ELISA (0.25–10 pg/mL: Erb, 2004). Surface Plasmon Resonance (SPR) is the most common label-free method used to date. This highly accurate optical sensing method measures an electron wave density charge phenomenon that occurs when the analyte under study binds to the receptor site and changes the refractive index of the metal film (Zhao et al., 2000). Sensitivities of 0.01–100 ng/mL have been achieved for an enhanced horseradish peroxidase (HRP)-catalyzed IFN-γ immunoassay (Kim et al., 2005).

**Design parameters for ideal in-flight sensor**

None of the typical methods have been appropriate for long-term, real-time monitoring of these and other biological species in space, however, mostly due to equipment size and volume constraints, and because they tend to be time-consuming, cumbersome techniques that require excessive astronaut interaction. Design parameters for an optimal in-flight biosensor are outlined in Table 4.

**MEMS and NEMS devices: are we there yet?**

Micro and nanoelectromechanical systems (MEMS and NEMS) offer a variety of advantages over contemporary sensor technologies and are being widely studied and developed for molecular diagnostic applications. These devices, besides being small and lightweight, consume low power, allow for on-chip electronics, and can be less invasive to the body, thus making in vivo monitoring feasible.

To date, MEMS sensors have been used for a variety of biomedical applications like the development of micropumps and microneedles for drug delivery (McAllister et al., 2003), the use of microchips for analysis of genetic markers of disease (Kopp et al., 1998), in the detection of DNA defects (Fritz et al., 2000), cardiovascular monitoring (Najafi and Ludomirsky, 2004), to mention a few. Some of the most recent bioassays figure microchips for detection of pathogens in urine (Kristo et al., 2003), microarrays for identification and profiling of carbohydrate-binding proteins (Galanina et al., 2003) and scale downs of the most traditional technique, micro-ELISAs (Sato et al., 1990).
Effects of temperature drift and non-specific binding. Applications availability to make a measurement comparing coated and uncoated surfaces. One advantage of this type of array sensor is the use of thiols and self-assembled monolayers (SAMs) on gold-coated substrates, allowing measurement of myoglobin for the purpose of rapid diagnosis of acute myocardial infarction (Arntz et al., 2003). The sensitivity achieved for myoglobin detection was found to be below the 20-μm/mL range. Other applications include incorporation of ion-selective SAMs to detect calcium ions in solution (Ji and Thundat, 2002) and glucose monitoring through glucose oxidase-coated microcantilevers (Pei et al., 2004).

Finally, in bimetallic mode, bending is caused by the difference in linear expansion coefficients between the silicon cantilever and the material used to coat it. This method makes it possible to measure very small changes in temperature in the order of 10⁻³ K (Lang et al., 2005). Berger et al. (1998) were able to quantify nanograms of CuSO₄ with relative resolution in the picogram scale using this method of thermal analysis.

Because of the large number of possible functionalities that can be given to them, cantilever systems will likely continue to have many applications in immunoassays. By changing the chemical group bound to the surface, a new sensor can be created utilizing functionalized surfaces. This biomolecular recognition technique has been used in DNA hybridization experiments and also in monitoring immunoglobulin binding. Another label-free protein assay has been created using nanocantilever arrays to allow measurement of myoglobin for the purpose of rapid diagnosis of acute myocardial infarction (Arntz et al., 2003). The sensitivity achieved for myoglobin detection was found to be below the 20-μm/mL range. Other applications include incorporation of ion-selective SAMs to detect calcium ions in solution (Ji and Thundat, 2002) and glucose monitoring through glucose oxidase-coated microcantilevers (Pei et al., 2004).

Microfabricated cantilever beams were first used as transducers with a functionalized tip (Afrin et al., 2004; Grandbois et al., 2005), and magnetoelastic immunosensors for E. coli assays (Ruan et al., 2003). Moulin et al. (2000) also used microfabricated cantilevers in resonant mode to measure surface stress caused by IgG and BSA. They were able to distinguish between compressive and tensile strength, and show the possible application of a sensor of this type for long-term monitoring of protein denaturing.

In static mode, the deflection of the cantilever is quantified through the use of a laser beam or via piezoresistive strain gauges. If only one side of the cantilever is coated, static deflection mode is usually used. For both sides, resonant frequency changes are measured. Fritz et al. (2000) have placed immobilized proteins on one side of microcantilever beams, and measured the deflection induced by the ligand binding in a fluidic environment as a measure of concentration. This technique does not require chemical tagging or fluorescence; therefore, it eliminates pretreatment steps used in regular assays. Gold-covered cantilevers aid in functionalizing the surface. This biomolecular recognition technique has been used in DNA hybridization experiments and also in monitoring immunoglobulin binding. Another label-free protein assay has been created using nanocantilever arrays to allow measurement of myoglobin for the purpose of rapid diagnosis of acute myocardial infarction (Arntz et al., 2003). The sensitivity achieved for myoglobin detection was found to be below the 20-μm/mL range. Other applications include incorporation of ion-selective SAMs to detect calcium ions in solution (Ji and Thundat, 2002) and glucose monitoring through glucose oxidase-coated microcantilevers (Pei et al., 2004).

Carbon nanotubes (CNTs) and nanowires (NWs) have emerged as powerful, ultrasensitive, electrical sensors for biological and...
chemical detection (Patolsky and Lieber, 2005). A few nanometers in diameter, typical CNTs and NWs exhibit high length-to-width ratios and are often referred to as 1-dimensional structures. Because of this arrangement, these nanostructures possess high rates of electron transfer with very low resistance, making them ideal for electronics and for use as transducing elements in, for instance, biosensing applications. CNTs are used to produce NWs of other materials such as gold or zinc oxide and these in turn can be used to produce nanotubes of other materials such as gallium nitride. Silicon NWs have been able to detect picomolar quantities of streptavidin (Cui et al., 2001). CNTs with carboxyl groups or hydrophobic functionalities have been used as AFM tips to study molecular interactions (Wong et al., 1998). Used as biomolecular probes with the ability to directly manipulate the sample, they have served as titers with acid and basic groups so that visual patterns can be created based on these molecular affinities. Modified CNTs with platinum nanoparticles have shown enhanced sensitivity and response time to plain carbon nanotubes for glucose sensing, as well (Hrapovic et al., 2004).

“Immuno-carbon nanotubes” have been created through functionalization with antibodies for the detection of the pathogen E. coli O157 (Elkin et al., 2005). CNTs have also been modified with quantum dots (Banerjee and Wong, 2004), DNA strands (Wang et al., 2004), amino acids (Georgakilas et al., 2002) and proteins (Karajanagi et al., 2004). Avidin and streptavidin-conjugated ZnSe/CdSe quantum dots have been incorporated to a nanowire through electropolymerization for sensing of biotin–DNA. This method has achieved nanomolar sensitivity and proven to be useful in showing that direct incorporation of biological molecules into conducting polymers has the advantage of site-specific positioning and ultrahigh sensitivity (Ramanathan et al., 2005).

Nanobelts (NBs) are similar to NWs but differ in that they are more structurally controlled, anatomically flat and have well-defined side surfaces (Wang, 2004). Though more recently developed than NWs (Pan et al., 2001), they present the potential for biosensing by being used as resonators (Bai et al., 2003), nanocantilevers (Hughes and Wang, 2003) and field-effect transistors (Wang, 2004). Some of the challenges that NBs face are the thorough understanding of their growth mechanisms, improving their selectivity for biochemical sensing applications, how to functionalize them, and how to integrate them with other nano and micromechanical components (Wang et al., 2004).

Optical sensing

Microscale optical sensors are based on the same detection principles of as traditional optical sensors: absorption, diffraction, refraction, fluorescence and interference. Fluorescence is perhaps the most commonly used principle for immunoassays. As previously mentioned at the introduction of the MEMS/NEMS section, the ELISA technique has already been miniaturized (Sato et al., 2004; Rossier and Girault, 2001) and has been used for the detection of interferon-gamma and D-dimer. Needless to say, applications of these lab-on-a-chip techniques can be extended to all the traditional research areas where ELISAs are used by changing the functionalized layer. Flow cytometry has also been miniaturized (Wang et al., 2004).

Surface plasmon resonance (SPR) and optical interferometry are methods based on measurement of differences in the refractive index of a sensing layer after the species of interest bind to the transducing element. In SPR, the affinity reaction on a functionalized gold layer is sensed by an evanescent beam of light and in interferometry, it is measured through the phase difference in two coherent light beams (Seydack, 2005). In the near future, SPR will most likely be miniaturized like the other aforementioned immunoassaying techniques. A few SPR applications include cancer diagnostics (El-Sayed et al., 2005; Campagnolo et al., 2004), detection of food pathogens such as Salmonella (Bokken et al., 2003) and E. coli O157:H7 (Subramanian et al., 2005), and proteomics (Yuk and Ha, 2005; Murphy et al., 2003). One interesting interferometric application is what is referred to as “smart dust”: nanostructured porous particles that exhibit a specific reflective pattern when probed with a remote laser. Though mainly intended for identification of aerosolized biochemical species such as those used in biological warfare, this platform is suitable for future nanoscale immunosensors because silicon can be easily functionalized with antibodies or other affinity species (Schmedake et al., 2002).

Fluorescent dyes and nanoparticles have been linked to biological species of interest and coupled with some of the techniques mentioned in the previous sections to enhance sensitivity and allow detection. Luminescent nanoparticles have been shown to have better photostability and sensitivity than organic dyes (Santra et al., 2004). The nanoparticles used for probing and labeling include quantum dots, colloidal gold and silver, polymer beads, dye-doped nanoparticles, and organic nanoparticles (Seydack, 2005). Out of these, quantum dots (which are semiconducting light-emitting nanocrystals) and polymer beads have been widely used in the study of cancer (Gao et al., 2004; Sage, 2004), bacteria (Wenhua et al., 2004), immunological and cellular interactions (Michalet et al., 2005; Stpiatura et al., 2004; Yavuz and Denizli, 2003), and multiplexed detection.

Useful and more detailed reviews are presented by Gauglitz (2005), Rich and Myszka (2005) and Marazuela and Moreno-Bondi (2002) on these and other optical biosensing techniques.

Polymers for biochemical detection

Polymers have been used in many applications such as drug delivery, bioseparation of proteins, tissue engineering and immunoassays (Roy and Gupta, 2003; Miyata et al., 1999). One technique that is likely to be commercialized for the improvement of immunoassays is the use of molecularly imprinted polymers (MIPs). Molecular imprinting is a technique used for functionalizing polymers with synthetic binding sites that are specific to a species of choice. The key to this specificity is to mix the analyte of interest while polymerization is occurring. After full polymerization and elimination of the template are accomplished, the resulting polymer is left with functional groups that are highly selective that will only bind to the chemical template it was previously treated with; therefore, the
use of the word ‘imprinted’, as it leaves a chemical impression on the polymer.

Because of their high sensitivity and chemical and mechanical stability, molecularly imprinted polymers are highly appropriate for harsh chemical environments such as those found within biological systems and could ideally be used in a large number of tests, without significant losses in affinity and selectivity. Polyelectrolytes, polystyrenes, and polynamethacrylates have been used for solid phase extraction, and also tested in the monitoring of polycyclic aromatic hydrocarbons in water (Dickert et al., 2000). Molecularly imprinted sorbent assays (MIAs) allow for differentiation of compounds within the same chemical family, i.e. corticosteroids (Haupt and Mosbach, 1998a), with almost no cross-reactions with competitive ligands (Ramström et al., 1996 as cited by Haupt and Mosbach, 1998a). One of the biggest challenges encountered in the preparation of these polymers is the presence of polar solvents such as water minimizes the interaction between the MIP and the ligand, thus losing affinity and selectivity (Allender et al., 2000). Current medical research has taken between the MIP and the ligand, thus losing affinity and selectivity (Allender et al., 2000). Other species such as theophylline have proven useful to allow transcutaneous monitoring of analytes, and have thus been considered for future non-invasive monitoring of drug levels in blood.

With all of these capabilities, MIPs have the potential to be coupled to any of the aforementioned micro/nanostructures as a way of functionalizing to create specificity toward an analyte or enhance the sensitivity of a transducing element.

Quartz crystal microbalances (QCMs)

Another sensor with extensive biosensing applications is the quartz crystal microbalance. A QCM is a thin quartz disc coated with a metal such as gold (which acts as an electrode) that vibrates at a specific frequency when current is applied. The frequency of the quartz is linearly related to the deposition of mass on its surface, assuming a rigid, homogeneous thin layer is formed. This linear behavior between the change in frequency and the amount of material deposited on the surface of the crystals has been studied extensively since the late 1950s and is characterized by the Sauerbrey equation (Collings and Caruso, 1997). While the sensitivity of a QCM (5 MHz) is in the 0.5-ng/mL range, thus comparable to optical methods such as SPR (Köbleringer et al., 1995), the size of the sensor is large enough to allow for extensive study of the challenges of surface chemistry at a macroscale. Its ease of handling in a lab setting and cost effectiveness (Skládal, 2003) makes it an excellent platform that helps to reduce the number of confounding variables in the design of microdevices. Some of the challenges that QCMs face are mostly related to the stability of the functionalized layer, the development of high-throughput capability and noise in the raw data. QCMs were initially used in space for dust deposition monitoring in the Space Shuttle robotic arm. Their main use at this time, however, has been in ground-based applications such as studies of biomolecular adsorption, molecular and antibody–antigen interactions and immunoassays. A summary of these and other applications is given in Table 5.

Table 5
Summary of biosensing QCM applications

<table>
<thead>
<tr>
<th>Application</th>
<th>Analyte</th>
<th>Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td>Muratsugu et al., 1993</td>
</tr>
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<td>Cortisol</td>
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<td>10×10⁶ cells/mL</td>
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Despite all the ground and in-flight research performed to elucidate how the immune system is affected by the conditions of space flight, there is still a need for real-time analysis in microgravity conditions. One way to solve this problem is to design a gravity-independent biosensor with the capability of analyzing biochemical markers. Selecting the appropriate markers that provide useful information about the state of health of a crewmember poses a dilemma. Candidate biomarkers exist in most body fluids, but blood serum tends to be fluid of choice for obtaining this physiological information. Choosing the right set of markers to determine immune response has been challenging, as a large number of them are multifunctional, working within different body systems. So far the markers of choice for both ground and flight studies have been a combination of cytokines, catecholamines, and other hormones. No one set of markers seems to provide enough information to extrapolate to the entire human body system, but rather point to immune cell functionality or lack thereof. Thus, to be able to accurately determine the nature of an immune response, or whether a crewmember is immunosuppressed, a biosensor with multi-analyte capability would prove most useful. Even though MEMS and NEMS devices are mostly in experimental stages, they possess the potential for ultra-sensitive analysis with minimal sample volume and with the capacity for parallel multi-analyte capability desired for the ideal in-flight sensor mentioned herein.

Concluding remarks

Table 5
Summary of biosensing QCM applications

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