Abstract: Salivary flow rate, immunoglobulin, and periodontal status were affected during a simulated Skylab mission. The effect is more prominent after long-duration space flights and can persist for several weeks after landing. The objective of this study was to determine the effect of a simulated Mars environment on periodontal status and levels of salivary microorganisms and immunoglobulins in the human oral cavity. Twelve healthy male volunteers were studied before, at 1 and 2 weeks, and after completion of a mission in an isolated, confined simulated Mars environment at the Mars Desert Research Station, USA. We conducted a current stress test, measured salivary immunoglobulin, cortisol, α-amylase, salivary flow rate, and levels of plaque and salivary microbes, and assessed clinical periodontal parameters (probing depth, bleeding on probing, and clinical loss of attachment). Salivary IgG levels and Streptococcus mutans activity were significantly higher at 1 week. Values for clinical periodontal parameters (probing depth, bleeding on probing, and clinical loss of attachment) significantly differed at 1 week. Stress might be caused by the difficulty of the mission rather than the isolated environment, as mission duration was quite short. Periodontal condition might worsen due to poor oral hygiene during the mission. The present findings show that all periodontal conditions and levels of oral bacteria and stress after completion of the simulated Mars mission differed from those at baseline. To verify the relationship between stress status and periodontal health in simulated Mars missions, future studies using larger patient samples and longer follow-up will be required. (J Oral Sci 55, 139-143, 2013)

Keywords: periodontal; saliva; isolated environment; oral microbes; immunoglobulin.

Introduction

During future space missions involving a space station or Mars mission, international crews will undertake complex activities over an extended period of time in isolated and extreme environments. Microgravity and isolated environments were found to affect human physiology (including that of the oral cavity) and the psychological status of astronauts during both short- and long-duration spaceflight. Health problems associated with such environments include bone loss, muscle atrophy, cardiac dysrhythmias, and altered orientation (1,2). Reports suggest that long-duration space flight will increase the risk of dental emergencies such as cracked teeth, inflammation or infection of tooth pulp, temporomandibular disorders, periodontal abscesses, and dental caries (3-14). In addition, saliva composition was changed and oral health was compromised during a simulated Skylab mission (3-4). Current evidence indicates that, during long-duration space missions, mission duration may affect the manner in which isolated environments affect the oral health of space crews. However, only one study has evaluated the effects of a simulated Skylab mission on the oral health of astronauts. Thus, the present study was designed to investigate the effects of a simulated Mars mission on oral health, including periodontal status, using a simulated Skylab mission (3) (with some modifications) at the Mars desert research station, USA (MDRS). The MDRS is an analog of the Mars surface...
habit and was constructed according to Mars reference mission guidelines for mission simulations (13).

**Materials and Methods**

**Subjects**
The 12 crew members were selected from Mars desert research stations (MDRS, UT, USA) by International Lunar Exploration Working Group EuroMoonMars. This experiment on human subjects was conducted in accordance with the Declaration of Helsinki and all procedures were carried out with the adequate understanding and written consent of the subjects. Informed consent was received from all crew members. Ethical permission was taken from local ethical committee, JBR Medicare Health and Research Education, Punjab, India (IRB #201201 JBR, 2011) additionally this study was reviewed and approved by Mars Society Scientific Committee, USA. A control group was not enrolled because the subjects acted as their own controls, before starting the mission. The average energy and calcium intake of the volunteers during the analog Mars simulation was 2,400 kcal/day (range 2,080-3,205 kcal/day) and 1,200 mg/day (800-1,700 mg/day), respectively.

**Clinical and laboratory analysis**
Because toothpaste and mouthwash were not allowed, oral care was done by rinsing with normal water and brushing with baking soda twice daily. Duration of sleep was approximately 7 h. Sampling was done at 7 AM before, at 1 and 2 weeks, and at mission completion (i.e., after 14 days). Intraoral samples were taken from each crew member between 7:00 and 7:30 AM before brushing, rinsing with water, and breakfast. Different samples (e.g., dental plaque and stimulated saliva) were taken before the mission, at 1 week, and at mission completion. Oral clinical examinations were done to establish the extent of dental plaque, calculus formation, and alterations in tooth, bone, gingiva, and other soft tissues. After collection of samples, one examiner recorded clinical periodontal parameters (probing depth, bleeding on probing, and clinical loss of attachment) for each crew member. Probing depth at six sites per tooth

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical periodontal characteristics of 12 healthy subjects during 14-day simulated Mars analog mission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Day 0</td>
</tr>
<tr>
<td>Probing depth (mm)</td>
<td>2.1 (1.1)</td>
</tr>
<tr>
<td>Clinical loss of attachment (mm)</td>
<td>1.6 (0.6)</td>
</tr>
<tr>
<td>Bleeding on probing (%)</td>
<td>4.32 (3.24)</td>
</tr>
</tbody>
</table>

Values are mean (SD)

Fig. 1 Anaerobic microbial counts (log_{10} of mean colony-forming unit count per mL × 10^-1) in stimulated saliva from 12 crew members during 14-day simulated Mars analog mission. A = Total anaerobes; B = Bacteroides; C = Fusobacteria; D = Candida; E = Veillonella

Fig. 2 Aerobic microbial counts (log_{10} of mean colony-forming unit count per mL × 10^-1) in stimulated saliva from 12 crew members during 14-day simulated Mars analog mission. A = Total aerobes; B = Neisseria; C = Lactobacilli; D = Staphylococci; E = Candida

Fig. 3 Streptococcal counts (log_{10} of mean colony-forming unit count per mg × 10^-1) in plaque from 12 healthy subjects during 14-day simulated Mars analog mission. A = Streptococci, B = S. sanguinis, C = S. mutans, D = S. salivarius, E = S. mitis

(mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) was measured using a manual probe (Hu-Friedy, Chicago, IL, USA). Clinical loss of attachment was determined by measuring inter-
proximal sites only. Samples were preserved at -4°C for further analysis. A wax carver was used to collect dental plaque from buccal, lingual, and proximal surfaces of two anterior and posterior maxillary and mandibular teeth. The samples were placed in a sterile tube. Mean (SD) weight was 2.8 (0.9) mg. Unstimulated saliva was collected, as described in a previous study (15). Stimulated saliva was collected using a saliva collection device (Versi-SAL, Oasis, Vancouver, WA, USA). Salivary flow rate was measured using a standardized technique (8). Serial 10-fold dilutions of samples were plated onto a variety of bacteriologic media, as described previously (14). The microbial levels of different microbes were analyzed as described previously (14). Immunoglobulin A, IgG, and IgM were measured in stimulated saliva samples, using ELISA kits (Biosource International, Camarillo, CA, USA). A current stress test was used to measure stress, as in our previous study (8). Salivary cortisol (Salimetrics Inc., State College, PA, USA) and α-amylase (Alpha-amylase assay kit, Salimetrics Inc.) were also measured.

Data analysis
The Student t test and ANOVA were used to analyze changes in microbial levels, clinical periodontal status, and biomarkers of salivary stress. Pearson correlation coefficients between periodontal parameters, salivary immunoglobulin, and microbes were also calculated. The data were analyzed using SPSS version (SPSS Inc 11.0, Chicago, IL, USA).

Results
Values for probing depth, clinical loss of attachment, and bleeding on probing were significantly higher at 1 week and mission end (P < 0.05, SD 10-12), as compared with baseline (before mission) (Table 1). We analyzed microbe levels in stimulated saliva (Figs. 1 and 2) and dental plaque (Fig. 3) collected during the mission. As compared with baseline, levels of total anaerobes, *Bacteroides*, *Fusobacterium*, and *Veillonella* were higher at 1 week and mostly remained so at 2 weeks (Fig. 1). In addition, levels of total aerobes, *Neisseria*, *Lactobacilli*, *Staphylococci*, *Candida*, and enteric bacilli were higher at 1 week as compared with baseline and were almost unchanged at 2 weeks (Fig. 1). The level of *Streptococcus mutans* was significantly higher as compared with baseline (Fig. 3, P < 0.001, SD 10-12). Salivary cortisol, α-amylase, and current stress scores were significantly higher at the end of the mission as compared with baseline (Table 2, P < 0.05). The level of *Streptococcus mutans* was significantly higher as compared with baseline (Fig. 3, P < 0.001, SD 10-12). Salivary cortisol, α-amylase, and current stress scores were significantly higher at the end of the mission as compared with baseline (Table 2, P < 0.05). Salivary IgG levels were significantly lower at 1 week as compared with baseline (Table 3, P < 0.001). *S. mutans* level strongly positively correlated with salivary IgG (r = 0.67). Also, salivary IgG positively correlated with clinical periodontal parameters (i.e., probing on depth, clinical loss of attachment, and bleeding on probing: r = 0.58, 0.56, and 0.69, respectively). *S. mutans* also strongly positively correlated with salivary cortisol and α-amylase (r = 0.76 and 0.78, respectively).

Discussion
We found that the periodontal status of the present crew members was compromised, perhaps because of stress or the use of baking soda, which may have compromised oral immunity, as indicated by decreased levels of salivary immunoglobulin and salivary flow and increased

Table 2  Current stress test (CST) scores and levels of salivary biomarkers from 12 healthy subjects during 14-day simulated Mars analog mission

<table>
<thead>
<tr>
<th>Variables</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CST</td>
<td>2.5 (0.23)</td>
<td>2.9 (0.4)</td>
<td>3.45 (0.67)</td>
</tr>
<tr>
<td>Salivary α-amylase (U/mL)</td>
<td>59.6 (24.2)</td>
<td>60.5 (23.1)</td>
<td>78.9 (23.6)</td>
</tr>
<tr>
<td>Salivary cortisol (µg/dL)</td>
<td>0.3 (0.1)</td>
<td>0.4 (0.1)</td>
<td>0.5 (0.1)</td>
</tr>
</tbody>
</table>

Values are mean (SD)

*P < 0.05 vs. first day

Table 3  Salivary flow, IgM, IgA, and IgG levels from 12 healthy subjects during 14-day simulated Mars analog mission

<table>
<thead>
<tr>
<th>Variables</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA (mg/L)</td>
<td>121 (21)</td>
<td>85 (18)</td>
<td>92 (22)</td>
</tr>
<tr>
<td>IgM (mg/L)</td>
<td>5.2 (1.3)</td>
<td>3.5 (1.4)</td>
<td>2.2 (1.7)</td>
</tr>
<tr>
<td>IgG (mg/L)</td>
<td>58 (24)</td>
<td>38 (17)</td>
<td>42 (20)</td>
</tr>
<tr>
<td>Salivary flow rate (mL/min)</td>
<td>18 (3)</td>
<td>14 (4)</td>
<td>16 (5)</td>
</tr>
</tbody>
</table>

Values are mean (SD)
microbial activity, as was the case in a simulated Skylab study (6). We found that periodontal status was significantly compromised as in Skylab study (6), possibly due to the side effects of baking soda or stress. Stress is an important risk factor in periodontitis, as shown in our previous studies (15-17). Stress is induced in an isolated environment and in an international, multicultural work atmosphere (16-19); however, the harsh environment might have caused stress during this mission. Decreases in immunoglobulin levels might have been due to compromised mucosal immunity (20). Salivary IgA, IgM, and IgG are indicators of the activity of the mucosal immune system and have been used to characterize the effects of many stressors. S. mutans activity was significantly increased in our subjects, as in a previous study (6), perhaps due to dietary factors (21). Increases in total anaerobes, Bacteroides, Fusobacteria, Veillonella, total aerobes, Neisseria, Lactobacilli, Staphylococci, Candida, and enteric bacilli activity could be due to the isolated environment and might further contribute to higher prevalences of periodontitis and caries (22,23). S. mutans activity strongly positively correlated with salivary IgG, possibly because IgG antibody activity in saliva is increased by greater S. mutans activity (24). The correlation might also be due to an immune system response to the high antigenic load from increased levels of S. mutans. It could also be a consequence of an increase in the amount of collected plaque. Indeed, the amount of total and other Streptococci was also higher at mission completion. Salivary IgG positively correlated with clinical periodontal parameters, perhaps because salivary IgG may have a protective role in periodontal disease, i.e., by minimizing loss of tooth attachment tissue, probing depth, and bleeding on probing (25).

The major advantages of using saliva in this study were the relative ease of sampling, as sufficient amounts can be gathered for analysis. In addition, saliva is easy to store and cost-effective as compared with blood and urine samples (24). In addition, saliva sampling is noninvasive, painless, and straightforward. Thus, saliva can be collected under conditions in which blood collection would be difficult or inadvisable. Technological advances have improved experimental approaches, thereby eliminating limitations on using saliva as a diagnostic tool in monitoring health or disease status (23-27).

Our findings suggest that effects on periodontal parameters and oral immunity were increased in the simulated environment, which could be due to stress factors. Furthermore, reduction in salivary flow and Ig levels in saliva were probably responsible for increases in bacteria and yeast levels. In addition, the present findings may have broader clinical implications for individuals facing harsh conditions in particular environments. To verify the relationship between stress status and periodontal health in simulated Mars missions, future studies will need to use larger patient samples and a longer duration of follow-up.

Acknowledgments

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References