

Detection of biosignatures in Terrestrial analogs of Martian regions: Strategical and technical assessments

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Key Points:

- The strategy of Mars biosignature detection calls for more spatiotemporal and environmental characterizations of potential landing sites on Mars.
- We divide terrestrial Mars analogs into four categories based on different Mars geologic ages considering meteorite impact frequency, volcanic activity, and surface water content.
- We discuss various technologies used for Mars biosignature detection and their applications in different Mars analogs.

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Abstract: For decades, the search for potential signs of Martian life has attracted strong international interest and has led to significant planning and scientific implementation. Clearly, in order to detect potential life signals beyond Earth, fundamental questions, such as how to define such terms as "life" and "biosignature", have been given considerable attention. Due to the high costs of direct exploration of Mars, Mars-like regions on Earth have been invaluable targets for astrobiological research, places where scientists could practice the search for "biosignatures" and refine ways to detect them. This review summarizes scientific instrumental techniques that have resulted from this work. Instruments must necessarily be our "eyes" and "hands" as we attempt to identify and quantify biosignatures on Mars. Scientific devices that can be applied in astrobiology include mass spectrometers and electromagnetic-spectrum-based spectrometers, redox potential indicators, circular dichroism polarimeters, in situ nucleic acid sequencers, life isolation/cultivation systems, and imagers. These devices and how to interpret the data they collect have been tested in Mars-analog extreme environments on Earth to validate their practicality on Mars. To anticipate the challenges of instrumental detection of biosignatures through the full evolutionary history of Mars, Terrestrial Mars analogs are divided into four major categories according to their similarities to different Martian geological periods (the Early–Middle Noachian Period, the Late Noachian–Early Hesperian Period, the Late Hesperian–Early Amazonian Period, and the Middle-Late Amazonian Period). Future missions are suggested that would focus more intensively on Mars' Southern Hemisphere, once landing issues there are solved by advances in spacecraft engineering, since exploration of these early terrains will permit investigations covering a wider continuum of the shifting habitability of Mars through its geological history. Finally, this paper reviews practical applications of the range of scientific instruments listed above, based on the four categories of Mars analogs here on Earth. We review the selection of instruments suitable for autonomous robotic rover tests in these Mars analogs. From considerations of engineering efficiency, a Mars rover ought to be equipped with as few instrument assemblies as possible. Therefore, once candidate landing regions on Mars are defined, portable suites of instruments should be smartly devised on the basis of the known geological, geochemical, geomorphological, and chronological characteristics of each Martian landing region. Of course, if Mars sample-return missions are successful, such samples will allow experiments in laboratories on Earth that can be far more comprehensive and affordable than is likely to be practicable on Mars. To exclude false positive and false negative conclusions in the search for extraterrestrial life, multiple diverse and complementary analytical techniques must be combined, replicated, and carefully interpreted. The question of whether signatures of life can be detected on Mars is of the greatest importance. Answering that question is extremely challenging but appears to have become manageable. Keywords: biosignature; detection framework; extraterrestrial life; evolutionary history of Mars; field trial; instrument; Mars analog

1. Introduction

Mars exploration has become a trending worldwide goal of planetary missions in recent years. Multiple space agencies, such as the

Correspondence to: W. Lin, weilin@mail.iggcas.ac.cn Received 20 APR 2022; Accepted 22 JUN 2022. Accepted article online 16 AUG 2022. ©2022 by Earth and Planetary Physics. National Aeronautics and Space Administration (NASA), European Space Agency (ESA), China National Space Administration (CNSA), Roscosmos, Japan Aerospace Exploration Agency (JAXA), Indian Space Research Organisation (ISRO), and United Arab Emirates (UAE) Space Agency, act as primary participants in this global mission. Among scientific objectives in space exploration, life detection is one of the most crucial themes that facilitate understanding of habitability and life distribution in the universe. As a cutting-edge research realm in space and planetary sciences, astrobiology is a multidisciplinary study that focuses primarily on the habitable potentials and signs of life in both extraterrestrial and terrestrial environments (Lin W et al., 2020; Hallsworth et al., 2021). On this basis, many countries have valued and supported the development of astrobiology.

Missions to Mars have shifted from attempting to detect signs of life directly, to the more indirect approaches of searching for water and bioessential nutrients, then to characterizing potentially habitable environments, and now back to searching for signs of life (Farley et al., 2020; Smith, 2021). The initial and the key step to detecting extraterrestrial life is to differentiate between abiotic and biological signals. Indicators of the presence of biological processes, either extant or extinct, are termed "biosignatures"; these are the signals that Martian life exploration quests are attempting to detect. Potentially positive results from Viking biological experiments (Klein et al., 1976; Levin and Straat, 1977) and several arguable microbial biomorphs (e.g., magnetite nanoparticles and carbonate globules) observed in Mars meteorites (McKay et al., 1996; White et al., 2014) imply putative organized chemical reactions and closed compartment structure on Mars. However, to date no concrete evidence has ruled out all probabilities of abiotic derivation or Earth-source contamination. In the absence of ambitious in situ life experiments, habitable properties such as surface hydrous minerals and subsurface water-ice inventories have been found to be expansive on Mars (Carter et al., 2013; Wu X et al., 2021; Liu Y et al., 2022). Although carbon and nitrogen, indigenous and available bioessential elements, have been detected and corroborated in Martian soils, their distributions are not widespread nor are they abundant (Stern et al., 2015; Bridges et al., 2019; Koike et al., 2020). How to spot viable, dormant, or fossilized life is the biggest challenge confronted by astrobiologists. Given the success of the landing of Mars Perseverance and Zhurong rovers in 2021 and the ongoing development of ExoMars, the world's planetary science groups have been continuing to make progress in further astrobiological investigations on Mars.

1.1 Life and Biosignatures

To explore signs of life on Mars, the foremost question is "what is life?". Most of the current definitions of life, such as NASA's definition, "a self-sustaining chemical system capable of Darwinian evolution", may be too general for comprehensive exploration on other planets (Horowitz and Miller, 1962). In this most-frequently quoted definition, the criterion of self-sustainability excludes viruses, viroids, and prions from consideration; the other criterion, that a life form must be capable of Darwinian evolution, has an implicit terrestrial historical perspective that excludes evidence of isolated single organisms and would seem to reject data from any short-period observations (Luisi, 1998; Cleland and Chyba, 2002). Despite its attempt to be both necessarily restrictive but also open-minded, NASA's definition of life is not easily applicable due to the difficulties of assessing the self-sustainability, heredity, and evolution of Martian "life" (Luisi, 1998).

One of the definitions of life summarized by Carl Sagan consisted of five aspects: physiological, metabolic, biochemical, genetic, and thermodynamic (Bedau and Cleland, 2010). However, some of these definitions were proposed based only on what was then known about terrestrial life; others could be found in natural processes but also possibly in the behavior of man-made devices (Luisi, 1998; Bedau and Cleland, 2010). During the origin and evolution of life, at least on Earth, there is widely assumed to have been a transitional period (or prebiotic stage) in which non-living materials begin to exhibit the characteristics of "life" (if life on Earth was indigenous and not sourced through panspermia). Understanding the differences between life, non-life, and prebiotic molecules is crucial to identifying biosignatures beyond Earth. From the perspective of chemistry and evolutionary science, Pross argued that life is the entity capable of maintaining a high-energy state by constantly supplying additional energy (Pross, 2016). Accordingly, rather than differentiating living and non-living entities, focusing on the complexity of chemical constitutions and interactions detectable on other planets may be a more practical objective, at least at this time, when the origin(s) of life on Earth remains an open question.

In any case, we must acknowledge that life on other planets may differ significantly from terrestrial life. However, most definitions of life agree that living organisms, in order to grow, reproduce, and adapt to changing environments, must be able to consume nutrients and assemble them into complex functional molecules (Luisi, 1998). Although the most robust and resilient elements known to date to be used for macromolecule building are still the terrestrial bioessential elements (i.e., carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur), silicon and arsenic are potential substituents for carbon and nitrogen, respectively (Norris, 2007; Wolfe-Simon et al., 2009; Petkowski et al., 2020). Such considerations require that the search for signs of life should be less biased toward known terrestrial life forms, with regard to spatial distribution patterns of chemical elements and their relationships with microstructures.

Biosignatures are proxies for life that are capable of being measured directly by scientific instruments. Different categories of biosignatures have been proposed, including but not limited to those associated with biominerals; biofabrics; complex organic molecules; biogenic gases; chemicals that have spatial, chiral, isotopic, or temporally altered characteristics associated with life; and morphologies characteristic of cellular and extracellular activity, such as organic-containing compartment structures and extracellular polymeric substances (Marais et al., 2008; Summons et al., 2011; Hays, 2015).

The ideal biosignature would be one that is convincingly associated only with living organisms or with biological activities. However, various physical and chemical processes can occasionally generate signals similar to those associated with life. Thus, to be convincing, a claim that life has been detected will need to be supported by multiple biosignatures — ones that are compatible, diverse, and complementary enough that no set of abiotic natural processes could plausibly have resulted in the observed cluster of biosignatures (Marais et al., 2008). Nevertheless, practical engineering concerns demand that a Mars rover be equipped with as few instrument packages as possible. Thus, the set of portable instruments on a Mars rover must be chosen very carefully, and matched to each proposed landing region on Mars. The agnostic biosignature theory suggests that, to avoid misinterpreting any detected results, no particular molecular structure or evolutionary endpoint should be presumed (Cabrol, 2016; Cronin and Walker, 2016). Thus, a systematic framework of biological signal confirmation should be applied if a discovery of indigenous Martian life is to be supported convincingly. Additionally, it has been proposed that not just biosignatures but also anti-biosignatures should be sought, in order to exclude any signals that decisively indicate no presence of biological activity (Zahnle et al., 2011). If no signs of life are observable when redox imbalances are available that can easily direct resource-to-energy flows in a living system, anti-biosignatures are considered to be a likely explanation. However, few if any convincing anti-biosignatures have been identified at this time.

1.2 Mars Analog Environments

In order to study habitability and potential life signals on Mars, collecting first hand data from direct explorations on the Martian surface or in the Martian atmosphere would undoubtedly be the ideal approach. However, the extremely low accessibility to Mars and high cost of Mars missions block most scientists from such direct measurements. Therefore, numerous scientists have shifted their focus, launching Mars science projects on Mars-like regions on Earth. These Mars-like terrestrial environments and landforms are termed Mars analogs, identified by their similarities to the known or presumed geological, chemical, mineralogical, and/or ecological conditions of past or present Mars (Gómez, 2011).

The Red Planet, like Earth and other planets, has experienced a dramatic evolutionary history of climate for ~4.5 Ga, from early wetter to current freezing-arid conditions (Mangold, 2021). Many terrestrial Mars analogs are thought to be comparable to the wetter and more habitable early Mars; other places on Earth appear to be analogs of Mars when it was in transition, progressively becoming a reddish, drier, and less habitable planet. Terrestrial Mars analogs further help astrobiologists by providing places where scientific instruments can be evaluated for their likely operability and reliability on Mars. Scientific instruments are scientists' "eyes" and "hands" to determine geological and environmental properties and potential life signals. Combined investigations of a myriad of portable biosignature detection-related instruments in Mars-like places on Earth will improve the reliability and authenticity of potential future discovery of Martian life, since any findings suggestive of genuinely native Martian life will need to be supported by multiple and complementary pieces of evidence (Green et al., 2021).

Fairén et al. (2010) identified a number of representative Mars analogs based on three main Mars climatic stages, by reviewing the geological, geochemical, and biological characteristics of these analog environments. Instead of Mars analogs, Westall et al. (2015) depicted possible abiotic and prebiotic sources on Mars and related scenarios of how their Martian biosignatures might be detected. They put forward various reasonable hypotheses based on a series of bifurcated premises and suggested spatial occurrences of potential Martian biosignatures (Westall et al., 2015). Based on in-depth conference discussions, Hays et al. (2017) classified Mars analog environments in accordance with different types of terrestrial spaces; they focused on the preservation potentials of biosignatures in a wide range of Mars analog environments, including the advantages and disadvantages of each analog environment. They further elaborated how these places are suitable or unsuitable for recording biosignatures (Hays et al., 2017).

In this review article, we intend to discuss the potential for various different types of instruments to detect biosignatures in Marsanalogous settings; we will then propose a framework for biosignature exploration based on different locations and past or present environmental conditions of Mars. Therefore, first we introduce the major instrumental technologies available for biosignature detection, followed by their applications in different categories of Mars analogs, and we summarize current findings. We discuss not only instruments suitable for mounting on Mars rovers but also other potentially useful instruments. This review of instrument-based biosignatures and the priority of biosignatures is intended to facilitate future astrobiological studies beyond Earth.

2. Biosignature Detection Techniques

Representative instrumental technologies that either have been used or can be used to detect biosignatures on Mars are presented below. Certainly, multiple biosignatures can be observed by a single instrument or technique, and different instruments together can in turn validate the presence of a biosignature. To corroborate Mars life exploration results, the selected biosignatures must be sufficiently compatible, diverse, and complementary so that no natural non-biological processes could account for the detected and verified pattern. However, engineering considerations require that Mars rovers must be equipped with suites of instruments that do not exceed certain limits of weight and complexity. Hence, the set of portable instruments to be mounted on a Mars rover must be devised carefully, keeping in mind that sample-return missions are a promising way to expand the search for important signatures, by making it possible to use Earth-bound instruments that will not be subject to such constraints.

2.1 X-ray Spectroscopy

Spectroscopic techniques of high reliability and sensitivity require little or no sample preparation and thus are popular tools on Mars rovers, X-ray spectrometers in particular. The frequencies of light waves absorbed or emitted by atoms and molecules indicated specific, characteristic, electronic transitions. X-rays are photons of relatively high electromagnetic energy, strong enough to knock electrons out of their atomic orbitals. By analyzing the energies released from X-ray emission or absorbance by rock/soil samples or biomineralized products, their compositions — their major and trace elements — can be identified and quantified (Siegbahn, 1962). X-ray diffractometry (XRD) and fluorescence (XRF) are two prominent types of mineral analytical techniques. Using such techniques to characterize the types and chemical distribution patterns of surface minerals associated with previous knowledge about their potentials of organic or biosignature preservations, can suggest the degree of habitability that the sampled region might afford (Bish et al., 2014). XRD or XRF-based mapping is a useful technique for determining and visualizing mineral or elemental distributions. As discussed in the previous section, coupled to microscopy, X-ray spectroscopy can analyze sample constituents by focusing on a micro-scale field of view (Friel and Lyman, 2006). Synchrotron X-ray microprobe technique, commonly applied in Martian meteorite studies, can examine small sizes of metal-rich phases in minerals and is thus a useful tool for more detailed analysis of samples returned from Mars (Liu Y et al., 2021). X-ray absorption near-edge structure (XANES) elucidates changes in the electronic structure of atoms and the oxidation states of electrochemically active elements, such as nitrogen in organic matter (Koike et al., 2020). X-ray spectroscopy can be employed in various situations on Mars to resolve geochemical profiles.

2.2 Reflectance and Transmission Spectroscopy

Reflectance/absorption spectroscopy is a term describing numerous spectroscopic techniques that take advantage of patterns of light absorption by the measured materials; instruments of this sort are broadly loaded on rovers. Paired with fluorescence spectroscopy, these spectroscopes can measure electronic transitions from the ground state to the excited state and their reverse processes (Skoog et al., 2018). Based on the wavelength range applied, reflectance spectroscopy can be divided roughly into ultraviolet (UV)-visible, visible-near infrared (VNIR), short-wave IR (SWIR), mid-IR, and so on. Organic matter has unique absorption spectra that can be revealed by the use of these techniques, and thus can be distinguished from inorganic signals (Rossel et al., 2006; Gholizadeh et al., 2021). Fourier transform IR (FTIR) spectroscopy is an upgraded version of general IR spectroscopy that can more effectively detect minerals and organic functional groups. FTIR employs an interferometer to generate an interferogram; after Fourier processing, the raw IR spectral data are transformed into a more readable IR spectrum (Artz et al., 2008). However, because FTIR requires slightly more cumbersome sample preparation, FTIR is not currently feasible for in situ Mars exploration (Baker et al., 2014). Prototypes of mountable FTIR instruments have been developed since the 2000s (Anderson et al., 2005).

Transmission spectrometers measure the transmittance and brightness of the atmosphere in various wavelength ranges. Planetary atmospheric compositions can be determined by transmission spectroscopy. A change in atmospheric spectra is likely to indicate biogenic gas production, and thus can be a potential biosignature (Meadows et al., 2018). As a convenient global remote sensing technique, transmission spectroscopy can be useful for Martian atmospheric biosignature exploration (Tinetti et al., 2013). Considering the relatively inert tectonic, magnetic, and volcanic activities on Mars, detection of temporal variation in elemental ratios would be a signature strongly suggestive of biological effects (Connerney et al., 2005; Brož et al., 2021). However, such an observation would be unlikely except in the case of an active biosphere on a relatively large scale. To date, transmission spectroscopy has been applied only scarcely in Mars analog studies; we suggest more extensive trials in a wider variety of Mars analog environments to explore whether this biosignature might be combined with other techniques to extract a more sensitive, multifaceted, signal of possible microbiological activity. Likewise, trace gas detectors and gas isotope analyzers can achieve the same scientific goals (Khan et al., 2019; Jahromi et al., 2020).

2.3 Laser and Raman Spectroscopy

Laser spectroscopic techniques are practical on other celestial bodies due to their use of highly focused excitation sources that are quite resistant to external perturbations. A laser beam refers to a highly collimated bundle of light in a narrow wavelength range, from UV to far IR, that is amplified by stimulated emission of radiation. A laser's high energy can excite orbital electrons in atoms or molecules to a higher energy level from which they then decay, in a spontaneous emission of light. This property allows laser techniques to probe for specific molecular structures and detect specific chemical species from their characteristic decay spectra (Lin YW et al., 2003). Laser-induced fluorescence (LIF) is able to locate and quantify organic compounds (Fisk et al., 2003). Gas chromatography (GC)-coupled laser spectroscopy can vaporize rock surfaces by laser beams, followed by gas separation by GC, to determine the abundances of elemental isotopes by means of the resulting spectra (Seneviratne et al., 2016). Laser-induced breakdown spectroscopy (LIBS) is a powerful tool to effectively detect a large range of light and heavy elements (Xu WM et al., 2021). In the methodological realm of astrobiology, LIBS is generally associated with IR and Raman spectroscopies (Sobron and Wang AL, 2012).

Raman scattering (inelastic scattering of photons) induced by laser sources is a significant physicochemical technique that can be used to explore about 30 subtypes of Raman spectroscopy, such as morphologically-directed Raman spectroscopy, transmission Raman, X-ray Raman scattering, inverse Raman spectroscopy, micro-Raman (µRaman), and UV resonance Raman spectroscopy (UVRR) (Lee et al., 2021). Raman spectra are particularly sensitive to pigments (e.g., carotenoid and scytonemin) and can effectively locate peaks that are characteristic of organic matter, biomineralized materials, and various other minerals. Choice of the range of excitation wavelengths focuses a Raman spectrometer's sensitivity on specific minerals and organic compounds (Vítek et al., 2014). Raman spectroscopy is a powerful tool to investigate either relatively fresh organic materials or persistent molecular remains from past biological activity, such as mineralized materials and organics (Rull et al., 2017).

2.4 Mass Spectrometry

Mass spectrometry (MS) collects information regarding the massto-charge (*m/z*) ratio of samples to determine elemental species and compositions. Mass spectrometers are generally favored in Mars missions because of their strengths in analyzing intricate chemical structures and isotopes, which are helpful for understanding the complexity of a chemical, prebiotic, or even biological system. MS comprises a myriad of variants based on the methods of sample separation, ionization, mass to charge selection, ion detection, and tandem construction (Wieler, 2014). Liquid chromatography-, gas chromatography-, and capillary electrophoresismass spectrometers (LC-MS, GC-MS, and CE-MS) are capable of identifying organic compounds such as amino acids that are commonly detected in the Solar System (Aerts et al., 2020). Ion chromatography (IC) is a high-performance LC (HPLC) that can quantify and separate soluble ions in soils, sediments, and liquid samples. However, due to the difficulty in bringing operable liquid water and chemical reagents to Mars, no HPLC instrument sufficiently versatile to detect amino acids is practical for current Mars missions, but IC remains an important tool for use in samplereturn missions (Glavin et al., 1999). The relative levels of perchlorate and chlorate to sulfate and chloride measurable by IC are known indicators of biological consumption of perchlorate and chlorate as preferable electron receptors, compared to sulfate (Jackson et al., 2012). GC-MS separates elements in the gaseous phase and measures their isotopic compositions (Ziolkowski et al., 2013). Isotope ratio mass spectrometry (IRMS) is specifically focused on isotope ratio determination. When associated with an elemental analyzer (EA), IRMS can allow the elemental composition of a sample to be determined in detail. Secondary ion mass spectrometry (SIMS) analysis can measure multiple isotope ratios, and obtain detailed elemental and molecular information (Mari et al., 2019). Although the use of in situ SIMS was suggested relatively early (Inglebert et al., 1995), SIMS spectrometers have not been loaded on Mars rovers but can be used for sample-return or laboratory-based projects (Vicenzi and Eiler, 1998). Mass spectrometers for isotopic analysis can be applied almost universally in all Martian environments since isotopic signatures are independent of postulated biomorphic traits, genetic materials, and organismal compounds.

2.5 Electrochemistry and Calorimetry

Electrochemical experiments require a wet chemistry apparatus that provides an aqueous setting; this extra requirement brings significant challenges to the Mars rover design, that, to date, have resulted in just one trial (on the Mars Phoenix mission). Nevertheless, electrochemical characterization remains an extremely important technique of enormous promise in the search for signals of present or past life. Although the presence of redox potential cannot alone be a biosignature, it indicates the availability of energy sources (one of the essential habitability factors) for, or active metabolisms of, life. Electrochemical (micro) calorimeters, IR thermometers, and oxidation/reduction potential (ORP) indicators are useful for determining the redox potential of various ion pairs of different valence states, which can be energy sources for life (Koeppel et al., 2019; Carrier et al., 2020). Electrochemistry and calorimetry can help to determine possible active metabolisms, based on microbial metabolism-driven nano-vibrations and waste heat, that are distinguishable from abiotic redox conditions (Neveu et al., 2018; Johnson et al., 2019). Examinations of alterations in energy flow, or disequilibrium redox reactions at odds with abiotic processes, could be an advantageous and constructive approach to support other evidence of possible biological activity. However, electrochemistry and calorimetry as prospective energy source indicators are useful only when life in analyzed samples stays active and is not virus-like.

2.6 Polarimetry and Circular Dichroism Spectroscopy

To examine the chirality of molecules, polarimeters and circular dichroism spectrometers are useful and sensitive devices (Gogoi et al., 2021). Homochirality (100% enantiomeric excess) is a strong indicator of a chemical system assembled in a delicate and organi-

zational manner (Blackmond, 2010). Most likely, incomplete enantiomeric separation instead of homochirality can be observed on Mars and thus the enantiomeric excess should be sufficiently high to rule out abiotic processes. Previous research has studied the application of polarimeters and circular dichroism spectrometers to Mars (Sparks et al., 2005, 2012), but these instruments have not been mounted on Mars rovers to determine local light polarization. Polarimetry and circular dichroism spectroscopy are supportive techniques for organic compound detection, since the enantiomeric excess or homochirality of organic molecules, instead of inorganic minerals (Pattrick et al., 2002), is an indicator of prebiotic processes or even biological metabolisms (Sharma, 2020; Shen JX, 2021). Generally with lower priority, these techniques have not been used in earlier Mars orbiting or roving missions. The remote imaging polarization method was employed during the favorable opposition of 2003, using a ground-based very large telescope; no evidence of circular polarization was found in observable locations within the investigation's limited spatial resolution and wavelengths (Sparks et al., 2005). Space-based remote circular polarizers on orbiters are suggested to be developed in the future to detect whether chiral signatures are present (Sparks et al., 2012).

2.7 *In situ* Nucleic Acid Sequencing and Microbiological Techniques

Almost all living organisms on Earth are nucleic acid-based. If Martian life shares a similar genetic system, nucleotide staining and sequencing techniques could largely facilitate life detection on Mars. Acridine orange (AO) selectively binds to and stains nucleic acids, resulting in an indicator of the presence of potential biogenic signatures (Fisk et al., 2003). The fourth generation of DNA sequencing, based on Nanopore technology, has been developed and supports in situ sequencing (Payne et al., 2021). Under even a thin layer (a few millimeters) of Martian regolith simulants, microbes can be protected from strong irradiation by the shielding effect (Gómez et al., 2010). Lichens have been shown to be capable of resisting Mars-like severe conditions (Sánchez et al., 2012). Cell culture techniques can also be used to attempt to grow any dormant Martian microorganisms; polymerase chain reaction (PCR) can be used to improve the success rates of attempts to detect and sequence any nucleic acids in Mars samples. In moister regions of Mars, such as hydrous mineral fields and water-ice layers, nucleic acid sequencing and microbiological techniques are potentially useful since viable microorganisms might be present locally. These techniques should be easy to conduct in situ on Mars but to date have not been implemented. It is important to test the sensitivity and reliability of these microbiological techniques in association with sample-return biological experiments, and for possible implementation on robotic rovers for future Mars in situ investigations.

2.8 Imaging and Visualization

Telescopes and microscopes are used to capture high-resolution images, including but not limited to possible targets for other instruments. Microscopes are too fragile to undergo the stresses of launching and landing but can be valuable tools for analyses of returned Martian samples. High quality cameras with panoramic and stereoscopic views can magnify images sufficiently to observe microstructures and microtextures characteristic of possible microfossils. In laboratory settings, scanning electron microscopy (SEM) is commonly used to detect microbial structures and to distinguish cellular structures and microbial fossils within soils, minerals, and rocks. An electron probe microanalyzer (EPMA) works similarly to an SEM (Chemtob et al., 2010). These microimagers are usually associated with an elemental microanalytical technology such as qualitative energy dispersive X-ray spectroscopy (EDS), wavelength dispersive spectroscopy (WDS), and electron backscatter diffraction (EBSD) for organic detection and elemental identification and guantitation (Cámara et al., 2016). Furthermore, imagers can also couple with mass spectrometry to dissect isotope compositions and metabolites (Newman et al., 2016). Fluorescent microscopy is a type of imaging technique that couples illumination to imagers for fluorescent substance observation (Bushby et al., 2012). Besides, imagers can be used in conjunction with many other instruments, such as mass spectrometers and thermometers. Due to the difficulty of locating microstructures or chemical indications of possible extraterrestrial life, efforts to predict possible visual signatures of such life and to search systematically for them are expected to be one of the last steps in efforts to detect life on Mars and other planets.

In general, instruments that can detect convincingly authentic organism morphologies (such as enclosed compartment structures, and organismal and trace fossils), organic molecules (reliable when abundant), and biogenic gases (such as methane, hydrogen, oxygen, carbon dioxide, and nitrous oxide) will be crucial in producing diverse evidence of high reliability in support of the presence of biological signals. Biofabrics and isotopic signature detectors also promise to be useful and robust tools in Mars life exploration missions. Biomineralization and spatial chemical distribution analyzers can, independently, provide tentative evidence of life, identifying samples worthy of further analysis, and could act as complementary tools to confirm and fortify similar results based on other instrumental measurements (Summons et al., 2011).

3. Applications of Instruments in Mars Analogs

To simulate biosignature exploration on Mars, analytical instruments ought to be tested on robotic rovers, deployed here on Earth in a variety of Mars analog environments with different habitability potentials, to verify their versatility and sensitivity in detecting and identifying biosignatures of life forms adapted to the various Martian environments (Heller, 2020). Mars, between its formation and now, has not always been a freezing and hyperarid planet. Accordingly, astrobiological studies of Mars must not focus solely on modern Mars but must look for signs of life possibly remaining from ancient, wetter Mars.

The Pre-Noachian Mars was almost entirely obliterated by later geological activities and impact events; this review thus does not attempt to classify any terrestrial environment as analogous to any Pre-Noachian region of Mars. We do identify representative terrestrial Mars analogs, and group them into four period-based categories based on the range of humidity conditions on Mars over its geological history: the Early-Middle Noachian Period (4.1-3.8 Ga), the Late Noachian-Early Hesperian Period (3.8-3.4 Ga), the Late Hesperian-Early Amazonian Period (3.4-(2.1-1.4) Ga), and the Middle–Late Amazonian Period ((2.1–1.4) Ga-present) (Figure 1). Inspired by Fairén et al. (2010), this article takes into consideration, besides average humidity, bombardment rates, volcanic activity, and extreme aquatic environments. Thus we identify and describe more Mars analog sites and classify them based on one more stage of the geological history of Mars. We note that multiple landforms can occur in the same geographical region, and here we roughly take a vast Mars analog region as a whole in the classification system. As pointed out by Hays et al. (2017), different landforms, such as subsurface environments, subaqueous environments, iron-rich environments, and hydrothermal springs, can be analogous to their respective Martian counterparts of multiple Martian periods. Some analog settings discussed in this review may include multiple and diverse



Figure 1. Global map of representative terrestrial Mars analogs in accordance with different Mars geological periods. Detailed information can be found in Table 1.

landforms that represent different niches on Mars. Herein, we enumerated some major representative Mars analogs on Earth (Table 1).

Determining the applicability of instruments to a life-detection Mars mission is paramount. Accordingly, this section reviews, in chronological order, previous assessments of instrument suitability, by type of instrument and instrumental technique, and suitability in studying samples from each Martian period and climate. Representative instruments and the results of their *in situ* robotic rover tests if available are listed below (Table 2). With several exceptions, most of these instruments have been mounted and tested on Mars rovers (Table 3).

3.1 Early-Middle Noachian Mars

3.1.1 Mars analog environments

Early–Middle Noachian Mars (4.1–3.8 Ga) was subject to frequent impacts that heated the Martian surface and melted subsurface ice inventories; with the formation of phyllosilicate clay minerals, Mars could have been humid and warm during this period (Abramov and Mojzsis, 2016; Palumbo et al., 2020).

Hadean-Early Archean Earth, prior to the origin of life or with limited life coverage, is an analog of Early–Middle Noachian Mars (Bosak et al., 2021); both suffered from frequent meteorite bombardments (Table 1) (Abramov and Mojzsis, 2008; Marchi et al., 2014). Greenstone belts in Pilbara (Western Australia) and Barberton (South Africa) are modern terrestrial regions representative of some of Earth's earliest recorded lithospheric surfaces, and are thus analogs of early Mars (Westall et al., 2011a, 2011b) (Table 1).

3.1.2 Applications of instruments

SIMS-determined isotopic carbon evidence from early Earth environments has demonstrated that the earliest life on Earth appeared approximately 4 Ga ago (Brocks et al., 2005; Dodd et al., 2017). The atmosphere of early Earth is postulated to have contained CO₂, N₂, H₂S, SO₂, CO, H₂O, NH₃, CH₄, and H₂ (Green, 2011). Using HPLC, GC, or UV spectrophotometry, the Miller-Urey experiment and similar simulations of prebiotic Earth conditions were able to detect the formation of numerous organic molecules (e.g., alcohols, aldehydes, organic acids, nucleobases, amino acids, sugars) (Table 2) (Miller, 1953; Oro, 1965; Bar-Nun and Hartman, 1978). Accordingly, detection of these molecules may suggest the presence of primitive life, or conditions favorable to the origin of life, on other planets. Gaseous products known to be biogenic, such as organosulfur compounds (e.g., methanethiol), have been identified as reliable signatures of early life on Earth (Pilcher, 2003). Simple hydrocarbons — 2-methylhopanoids — are regarded as biosignatures of stress responses to ancient Earth life (Newman et al., 2016). Thus, scrutiny of atmospheric components, primitive

Table 1. Representative terrestrial Mars analogs and their similarities to Mars of diffe	ferent ages.
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Mars period	Geological time	Mars analog	Similarity	References	
Early–Middle Noachian	4.1–3.8 Ga	Early Archean Earth environments at Pilbara and Barberton	Heavy bombardments; primitive planetary structure	(Abramov and Mojzsis, 2008; Westall et al., 2011a, b; Marchi et al., 2014; Bosak et al., 2021)	
Late Noachian– Early Hesperian	3.8–3.4 Ga	Rio Tinto; Dallol ephemera pools		Acidic fluids; polyextreme conditions	(Fernández-Remolar et al., 2004, 2005; Belilla et al., 2019; Gómez et al., 2019)
		Hawaiian/Idaho volcanoes; African islandic volcanoes	Basaltic compositions; volcanic activities; semiarid	(Craddock et al., 2005; Ansdell et al., 2011; Morse et al., 2011; Hughes et al., 2019)	
		Haughton, Sudbury; Meteor meteorite craters	Meteoritic impacts in a cold semiarid region	(Glass et al., 2008; Osinski et al., 2020)	
		Drylands in Qaidam Basin, Namib, Negev, Ladakh, Laguna de Tirez, Laguna de Peña Hueca	Millions of years of aridification; Mars-like landforms	(Bourke and Goudie, 2009; Mormile et al., 2009; Zheng MP et al., 2009; Miao Y et al., 2011; Anglés and Li YL, 2017; Xiao L et al., 2017; Kong FJ et al., 2018; Thombre et al., 2018; Pandey et al., 2020; Yair et al., 2021)	
Late Hesperian – Early Amazonian	3.4–(2.1–1.4) Ga	Atacama Desert	Millions of years of hyperaridification; strong irradiation; highly oxidizing soils	(McKay et al., 2003; Navarro-Gonza'lez et al., 2003; Fernández-Remolar et al., 2013; Rasuk et al., 2014; Veblen et al., 2015; Cordero et al., 2018; Cubillos et al., 2018; Azua-Bustos et al., 2022)	
		Western Deserts in North America	(As above)	(Chan MA et al., 2004; Hereford et al., 2006; Lybrand et al., 2013)	
Middle-Late Amazonian	(2.1–1.4) Ga –present	Polar regions	Extremely cold dry valleys, caves, and subglacial water- ice lakes	(Hess et al., 1979; Duxbury et al., 2001; Doran et al., 2002; Dickinson and Rosen, 2003; Mellon et al., 2004; Monaghan et al., 2005; Wentworth et al., 2005; Bernhard et al., 2006; Hausrath et al., 2008; Pollard et al., 2009; Fountain et al., 2010; Jackson et al., 2012; Battler et al., 2013; Tebo et al., 2015; Aun et al., 2019)	
		Near space and space station	Harsh space conditions	(Smith, 2013; Cottin et al., 2017; Rabbow et al., 2017; Cottin and Rettberg, 2019; Lin W, 2020; Lin W et al., 2022)	

organic molecules, and isotope ratios may plausibly detect biosignatures of early life on Mars.

3.2 Late Noachian-Early Hesperian Mars

3.2.1 Mars analog environments

Late Noachian–Early Hesperian Mars (3.8–3.4 Ga) received fewer meteoritic impacts than during the Early–Middle Noachian Period.

Hesperian Mars was characterized by intense volcanic activities, surface fluids, and hydrated sulfate deposits (Peretyazhko et al., 2016). While Hesperian Mars was less humid than Noachian Mars, its surface remained wetter than that of Mars today (Andrews-Hanna and Lewis, 2011). Terrestrial analogs to this Martian geological age category are the most diverse and thus more debatable.

Table 2.	Representative terrestria	Mars analogs and their	relevant biosignatures and	l astrobiological investigations.

Mars period	Geological time	Mars analog	Biosignature	Instrumentation	Robotic test	Reference
Early–Middle Noachian	4.1–3.8 Ga	Early Archean Earth environments at Pilbara and Barberton	Amino acids; carbon isotopes; 2-methylhopanoids; organosulfur gas	GC; IRMS; LC; SIMS; UV; XRF	Prebiotic Earth simulation	(Miller, 1953; Oro, 1965; Bar-Nun and Hartman, 1978; Pilcher, 2003; Newman et al., 2016; Bosak et al., 2021)
Late Noachian– Early Hesperian	3.8–3.4 Ga	Rio Tinto; Dallol ephemeral pools	Amino acids; biogenic micro- textures; Nanohaloarchaea; nucleic acids; pigments; saccharides	EA-IRMS; GC-MS; mid-IR; protein microarray; Raman; SEM-EDS; sequencer; SIMS; VNIR	Mars Astrobiology Rio Tinto Experiment (MARTE)	(Stoker et al., 2003; Parro et al., 2005; Souza-Egipsy et al., 2006; Edwards et al., 2007a; Davila et al., 2008; Orgel et al., 2014; Loiselle et al., 2018; Gómez et al., 2019; Fernández-Remolar et al., 2021)
		Hawaiian/Idaho volcanoes; African islandic volcanoes	Amino acids; cellular structures; high carbon and phosphorus; nucleic acids; organic molecules; silica deposits	AO stain; LIF; Raman; SEM-EDS; SWIR; UVRR; VNIR	Pacific International Space left for Exploration Systems (PISCES); Hawaii Scientific Drilling Project (HSDP)	(Fisk et al., 2003; Schowengerdt et al., 2007; Chemtob et al., 2010; Seelos et al., 2010; Ryan et al., 2021)
		Haughton, Sudbury; Meteor meteorite craters	Microbial structures; micro-fossils; simple organic molecules	FTIR; μRaman; SEM; XRD	Haughton-Mars Project Research Station (HMPRS); Drilling Automation for Mars Exploration (DAME); <i>in situ</i> instrument trials	(Parnell et al., 2004; Glass et al., 2008; Pullan et al., 2008; Cockell et al., 2020)
		Drylands in Qaidam Basin, Namib, Negev, Ladakh, Laguna de Tirez, Laguna de Peña Hueca	Biolipids; cyanobacterial scytonemin	GC-MS; LC-MS; Raman	Rover-mounted radar tests	(Sun Y et al., 2019; Shaner et al., 2020; Cheng ZY et al., 2021)
Late Hesperian– Early Amazonian	3.4–(2.1–1.4) Ga	Atacama Desert	Amino acids; biolipids; chirality; chlorophylls; clay- rich subsurface; microbial colonies; nitrate isotopes; organic carbon isotopes; organomineralized structures; secondary metabolites	Circular dichroism; EA-IRMS; FTIR; GC- MS; Raman; SEM- EDS; sequencer	Viking replicate experiments; Mars cave detection program; rover tests	(Wierzchos et al., 2006; Wynne et al., 2008; Vítek et al., 2010, 2014; Ziolkowski et al., 2013; Cámara et al., 2016; Azua- Bustos et al., 2020; Azua- Bustos et al., 2022; Shen JX et al., 2022)
		Western Deserts in North America	Former aqueous environments; polyextremotolerant bacteria	Mid-IR; μRaman; sequencer; VNIR; XRD; XRF	Field Integrated Design and Operations (FIDO); <i>in situ</i> instrument trials	(Pullan et al., 2008; Bishop et al., 2011; Belov et al., 2019)

Continued from Table 2

Mars period	Geological time	Mars analog	Biosignature	Instrumentation	Robotic test	Reference
Middle–Late Amazonian	(2.1–1.4) Ga –present	Polar regions	Endolithic microbial content; nitrate isotopes; (per)chlorate concentrations; micro-structures; organic carbon and nitrogen and isotopes; quadruple sulfur isotope	EA-IRMS; ESBD; fluorescence microscope; FTIR; IC; Raman; SEM; sequencer; XRD	Rover tests; <i>in situ</i> instrument trials	(Wierzchos et al., 2004; Anderson et al., 2005; Edwards et al., 2007b; Nadeau et al., 2008; Pullan et al., 2008; Jackson et al., 2012; Faucher et al., 2016; Archer et al., 2017; Moreras-Marti et al., 2021)
		Near space and space station	Microbial survival from exposure experiments, aerosols, and outside of space stations	Cell culture plates; sequencer	NASA Space Biology Program	(Horneck et al., 2010; Castro-Wallace et al., 2017; de la Torre Noetzel et al., 2020; Santomartino et al., 2020; Blachowicz et al., 2021; Góes-Neto et al., 2021; Simpson et al., 2021; Liu J et al., 2022)

Table 3. Corresponding scientific payloads for instrument-based biosignature detections mounted on successfully landed Mars rovers.

Mars rover	Technology	Payload	References
Viking 1 and 2	Imaging; mass spectrometry; microbiological experiments; X-ray spectroscopy	360-degree cylindrical scan camera; GC-MS; interior compartment for biological experiments; XRF	(Soffen, 1977)
Pathfinder	Imaging; X-ray spectroscopy	Imager for Mars Pathfinder (IMP); Alpha-Proton X-Ray Spectrometer (APXS)	(Golombek, 1997)
Spirit and Opportunity	Imaging; reflectance spectroscopy; X-ray spectroscopy	Panoramic Camera (Pancam); Microscopic Imager (MI); Miniature Thermal Emission Spectrometer (Mini-TES); Alpha Particle X-Ray Spectrometer (APXS)	(Yen et al., 2005)
Phoenix	Electrochemistry; imaging; mass spectrometry	Microscopy, Electrochemistry and Conductivity Analyzer (MECA); Robotic Arm Camera; Surface Stereo Imager (SSI); Mars Descent Imager (MARDI); Thermal and Evolved-Gas Analyzer (TEGA)	(Shotwell, 2005)
Curiosity	Imaging; laser spectroscopy; mass spectrometry; X-ray spectroscopy	Mars Descent Imager (MARDI); Mars Hand Lens Imager (MAHLI); Mast Camera (Mastcam); Sample Analysis at Mars (SAM); Chemistry and Camera (ChemCam); Chemistry and Mineralogy (CheMin); Alpha Particle X-ray Spectrometer (APXS)	(Grotzinger et al., 2012)
Perseverance	Imaging; laser spectroscopy; Raman spectroscopy; transmission spectroscopy; X-ray spectroscopy	Mastcam-Z; Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC); SuperCam; Planetary Instrument for X-ray Lithochemistry (PIXL)	(Bhartia et al., 2021)
Zhurong	Imaging; laser spectroscopy; reflectance spectroscopy	Navigation and Topography Cameras (NaTeCam); Mars Surface Compound Detector (MarSCoDe); Multispectral Camera (MSCam)	(Zou YL et al., 2021)

Rio Tinto, one fluvial analog of the Late Noachian–Early Hesperian Mars, is an acidic (mostly pH 2–2.5) and ferric sulfate-rich river drainage basin in southwestern Spain. Its acidity is sourced from the leaching and oxidation of sulfide minerals. These minerals are transformed into secondary mineral deposits through weathering, similar to the process in the Meridiani Planum, Mars (Fernández-Remolar et al., 2004, 2005). Other Mars-analogous environments of this age, similar to Rio Tinto, are an acidic and hypersaline geothermal Dallol volcanic area of ephemeral pools in Ethiopia (Belilla et al., 2019) and an acidic crater lake, Laguna Caliente, in Poás Volcano, Costa Rica (Wang JL et al., 2022).

Similar to the igneous compositions of Mars, the basaltic fields in the semiarid Kilauea Volcano (Hawaii) and the eastern Snake River Plain (Idaho, USA) bear fine-grained olivine basalts formed by rapid cooling of magma enriched in alkali metal oxides but depleted in silica (Hughes et al., 2019). Associated with a less desiccated condition (~150 mm annual precipitation), the Ka'u Desert within Kilauea Volcano is a basaltic terrain analog of Late Noachian–Early Hesperian Mars (Craddock et al., 2005). Due to their geomorphic features and minimal vegetation, Haughton Impact Crater and Sudbury Crater in Canada, and Meteor Crater in the USA, are analogous to crater regions of the cold wet Late Noachian–Early Hesperian Mars (Table 1) (Osinski et al., 2020). Mars analog meteorite craters can imitate closely the subsurface ice and broken depth-graded textures on Mars (Glass et al., 2008). Other Mars-analogous volcanic regions on Earth are the Sainte-Rose Island and the Teide Peak, both in Africa (Ansdell et al., 2011; Morse et al., 2011).

On the Qinghai-Tibet Plateau, the highest and largest plateau of the world, the driest region, Qaidam Basin, is a Mars-analogous desert (Anglés and Li YL, 2017; Xiao L et al., 2017). The average altitude of the Qaidam Basin is ~3000 m; its mean annual temperature is generally 2-4 °C (Miao Y et al., 2011). The aridity index within the Qaidam Basin can be as low as 0.008-0.04 (in the hyperarid range) (Kong FJ et al., 2018); this aridification is a consequence of the uplift of the Qinghai-Tibet Plateau, on which, subsequently, many freshwater lakes have evolved into saline lakes and even evaporitic playas (Zheng MP, 1997). Landscapes of Qaidam Basin have gradually changed to a Mars-like geomorphology, including gullies, fluvial valleys, alluvial fans, polygonal structures, gravel deposition, pits, dunes, and yardangs (Anglés and Li YL, 2017; Xiao L et al., 2017). In addition to the deserts discussed above, many more terrestrial dry areas and associated evaporitic playa-lake systems are analogs of the humid-to-dry transitional Mars, including the Namib Desert in Southern Africa (Bourke and Goudie, 2009), the Negev Desert in Israel (Yair et al., 2021), the Ladakh in India (Pandey et al., 2020), and Laguna des Tirez and Laguna de Peña Hueca in Spain (Thombre et al., 2018) (Table 1).

3.2.2 Applications of instruments

An exhaustive SIMS investigation revealed well-preserved molecular biosignatures under the acidic oxidative surface of Rio Tinto (Fernández-Remolar et al., 2021). Organic materials - possible biosignatures — were isolated from inorganic substances at Rio Tinto, detected using EA-IRMS (Table 2). GC-MS and LC-MS were conducted to detect and characterize lipid biosignatures (Shaner et al., 2020; Cheng ZY et al., 2021). Miniaturized Raman spectroscopy was found to be able to detect carotenoids, scytonemin, and mycosporine-like amino acids (Table 2) (Edwards et al., 2007a). SEM analysis associated with EDS and GC-MS observed biologically-induced textural variations in Rio Tinto (Souza-Egipsy et al., 2006). Lichen (e.g., Circinaria gyrosa), Deinococcus radiodurans, and an acidophilic chemolithotroph species (Acidithiobacillus sp.) isolated from Rio Tinto were tested for their ability to resist strong UV irradiation (Gómez et al., 2010). Probes such as protein microarrays have been employed in Rio Tinto to detect nucleic acids, peptides, and saccharides (Parro et al., 2005). Polyextremophilic Nanohaloarchaea have been discovered in acidic and hypersaline Dallol ephemeral pools (Gómez et al., 2019), suggesting that similar microbial life may have survived on Mars as the planet became more arid.

Within the Hawaiian volcanic area, LIF has been employed to detect and locate organic biosignatures (Ryan et al., 2021). SEM-EDS further confirmed high abundances of carbon and phospho-

rus, suggestive of cellular structures, within Hawaiian subsurface basalts (Table 2) (Fisk et al., 2003). The Hawaii Scientific Drilling Project (HSDP) has included studies of the subsurface biosphere of Hawaiian basaltic terrains (Fisk et al., 2003). UVRR spectroscopy and AO staining were applied to these basalts to search for evidence of microorganisms by searching for amino acids and nucleic acids (Fisk et al., 2003). Analysis of compounds detected in meteorite crater regions can be complex. On the one hand, impact events may stimulate abiotic synthesis of some simple organic molecules. Appropriate dosages of local radiation can polymerize organic matter, increasing its complexity (Parnell et al., 2004). On the other hand, even moderate-sized meteoritic impacts may not completely destroy genuine biosignatures of life present prior to the impacts: biomolecules, fossilized remains, and active or dormant microbial life detectible by FTIR and SEM may survive after these events (Parnell et al., 2004; Cockell et al., 2020). Accordingly, it is plausible that extremophilic microorganisms, stable biomolecules, and fossilized structures might be detectable in regions of Mars dating to the Late Noachian–Early Hesperian.

3.3 Late Hesperian–Early Amazonia Mars

3.3.1 Mars analog environments

At this stage of Mars evolutionary history (3.4–(2.1–1.4) Ga), the planet continued to lose water and atmosphere; impacts were declining in frequency; but some small-scale local environments remained moderately wet (Kite et al., 2017; Hu RY and Thomas, 2022).

The Atacama Desert is a temperate hyperarid ecosystem located in northern Chile, South America, which contains a variety of landforms (e.g., barren lands, geothermal fields, high-altitude volcanic area, and salars). It is thus an analog of Late Hesperian–Early Amazonian Mars (Table 1) (Azua-Bustos et al., 2022). The hyperaridity of the central depression Atacama Desert is due to a "sandwich" geographic setting: high mountains, the Coast Range in the west and the Andes Mountains in the east, as well as a constant temperature inversion effect by the Pacific anticyclone/ Humboldt current, prevent moisture from reaching it (McKay et al., 2003). In austral summer, the UV index of the Atacama Desert can be more than 11 or even up to 20 (Cordero et al., 2018), which is within the highest risk level. Due to the irradiative photochemical reaction and the lack of precipitation (<2 mm/yr), the core Atacama soils are characterized by remarkably bulky nitrate and perchlorate inventories that are sourced from high irradiation-driven photolysis (Michalski et al., 2004; Ewing et al., 2007; Shen JX et al., 2019). From north to south, the aridity of the Atacama Desert progressively decreases (Navarro-González et al., 2003; Shen JX et al., 2021). El Tatio in the high-altitude Atacama is another representative Mars analog that demonstrates the presence of opaline silica deposits similar to ones discovered on Mars; silicified filaments of microorganisms are observed in many silica sinters of the El Tatio geothermal system (Ruff and Farmer, 2016). Moreover, A myriad of evaporitic salars (e.g., Salar de Atacama, Salar Grande, and Salar de Llamara) in the Atacama Desert are similar to evaporite-rich sedimentary deposits on Mars (Fernández-Remolar et al., 2013; Cubillos et al., 2018; Reid et al., 2021).

Deserts located in the Western USA include the Mojave, the Colorado, the Great Basin, the Sonoran, and the Chihuahuan (Table 1). The section of the Mojave Desert known as "Death Valley" is the driest region within this area and has recorded world record temperatures. These deserts are cold drylands characterized by oxidizing soils, iron concretions, playas, and minimal vegetation (Chan MA et al., 2004). Minimum and maximum temperatures in this region can be –15 and 49 °C, respectively, with mean annual precipitation about 34–310 mm (Hereford et al., 2006). Nitrate and perchlorate are present in the Mojave Desert (Lybrand et al., 2013).

3.3.2 Applications of instruments

As the "World Dry Pole", the Atacama Desert has attracted many studies related to the search for Martian life. A shallow subsurface clay-rich layer (at a depth of just 30 cm) was found via FTIR in the hyperarid core of Atacama (Table 2) (Azua-Bustos et al., 2020). GC-IRMS probes of Late Hesperian-Early Amazonian Martian soils may yield biosignatures of microbial activity, such as carbon isotopes of *n*-alkanes, glycolipids, and phospholipids, depending on their ratios and abundances (Ziolkowski et al., 2013; Shen JX, 2020). Stable isotope ratios of nitrate deposits determined by EA-IRMS are found to be a useful tool for understanding biologically involved nitrogen dynamics (Shen JX et al., 2022). Homochirality has been examined in the Atacama Desert as supportive evidence of biomolecules characteristic of single microorganisms (Azua-Bustos et al., 2022). By applying SEM-EDS, microbial and organomineralized structures have been targeted and analyzed under the microscope (Table 2) (Wierzchos et al., 2006; Cámara et al., 2016). A scientific rover was remotely controlled by a group of scientists and roboticists to explore fluorescence excited from biomolecules such as chlorophyll (Cabrol et al., 2007). Another rover operated autonomously in the Atacama Desert studied the influence of subsurface minerals and environmental variables on water availability and microbial community profiles (Warren-Rhodes et al., 2019). Atacama soils have also been used to test Raman spectroscopy for the detection of secondary metabolites and microbial colonies (Table 2) (Vítek et al., 2010, 2014).

By use of XRD, XRF, VNIR, and mid-IR on carbonate rocks in the Mojave Desert, a transformation from aqueous to arid environments similar to Late Hesperian–Early Amazonian Mars was detected and characterized (Table 2) (Bishop et al., 2011). Nitrate and perchlorate are present in the Mojave Desert as potential electron acceptors and redox potential energy sources (Lybrand et al., 2013). Bacteria resistant to widely varying temperatures, changing pH, and oxidizing salts have been discovered in the Mojave Desert (Belov et al., 2019). Such studies support efforts to detect biogenic macromolecules, biomineralized structures, enantiomeric excesses, and indicative isotope ratios in Late Hesperian–Early Amazonian Mars regions, where they could plausibly be potential biosignatures.

3.4 Middle-Late Amazonian Mars

3.4.1 Mars analog environments

The Middle-Late Amazonian Mars is characterized by decreasing volcanic activity, high radiation, hyperaridity, extreme cold, low

atmospheric pressure, and slow oxidation of iron-rich rocks (Bibring et al., 2006). In this era, the surface of Mars already appeared desolate.

Numerous ecotopes, especially the hyperarid and hypothermal polar deserts, in Antarctic and Arctic regions, are analogs of modern Martian polar areas and subsurface water-ice layers (Mellon et al., 2004). The precipitation in Antarctic dry valleys is generally 3-50 mm per year; temperatures range from -30 to -14 °C (Doran et al., 2002; Fountain et al., 2010). Due to the polar ozone hole, the UV index in Antarctica can rise up to 6-12 at various sites (Aun et al., 2019). Remarkable natural reservoirs of perchlorate and nitrate deposits have been detected in Antarctic Dry Valleys such as the McMurdo Valleys (Jackson et al., 2012). Antarctic dry valleys have been used as modern Mars analogs for studies of meteorite weathering and diagenetic minerals (Dickinson and Rosen, 2003; Wentworth et al., 2005). Various Antarctic niches are analogous to Martian sites likely to be promising for life detection studies. For instance, caves on Mars, sheltered from harsh surface conditions, are analogous to caves in Antarctic drylands (Tebo et al., 2015); Antarctic subglacial lakes like Lake Vostok (Duxbury et al., 2001) are analogs of those water-ice repositories buried under Martian regolith (Orosei et al., 2018; Lauro et al., 2021); ancient microbial signatures are well-documented in these terrestrial subglacial lakes (Greenfield et al., 2020). Similar to ice-covered regions in Antarctica, Arctic Martian analogs include the Golden Deposit (Battler et al., 2013), Axel Heiberg Island (Pollard et al., 2009), and Svalbard Volcanic Complex (Hausrath et al., 2008). They are characterized by glaciers, periglacial landforms, permafrost, and ice-wedge polygons similar to those found on Mars (Head and Marchant, 2003; Christensen, 2006; Soare et al., 2014).

Earth's near space (20-100 km altitude) region, and the space region above Earth between 350-450 km altitude (around space stations), are analogs of modern Martian surface environments, by virtue of being irradiative, hyperarid, extremely cold, and hypobaric (Table 1) (Cottin et al., 2017; Rabbow et al., 2017; Lin W et al., 2022). To date, these extreme environmental parameters have not been well-simulated anywhere on the surface of the Earth or in the laboratory. Due to methodological and technological developments in recent years, a growing number of studies have investigated the biological effects of near space and space environmental conditions. What has been learned about the survival strategies of microorganisms in such environments has not only suggested possible survival conditions of microbe-like life on the surface of Mars but also offers insights into the limits to life on Earth and the possibility of interplanetary transport of life forms (Smith, 2013; Lin W, 2020).

3.4.2 Applications of instruments

In Antarctica, *in situ* XRD spectroscopy has been applied to mineral characterization tests (Table 2). FTIR has been used to detect endolithic microbial communities in Antarctic rocks (Anderson et al., 2005). Raman spectroscopy has detected organic biosignatures in Antarctica (Edwards et al., 2007b). Organic carbon and nitrogen contents and their isotope ratios in Antarctic permafrost soils have been measured via EA-IRMS to assess the likely habitability of analogous ground ice environments on Mars

(Faucher et al., 2016). SEM-ESBD and sequencing techniques have been applied to inspect microbial morphological structures and community compositions (Archer et al., 2017). Furthermore, *in situ* trials of fluorescent microscopes have been successfully carried out to understand the viability of Antarctic endoliths (Wierzchos et al., 2004).

Nanopore genome sequencing was tested in Earth's near space and on the International Space Station (Table 2) (Castro-Wallace et al., 2017). We have learned that a significant portion of magnetotactic bacteria with magnetosomes can survive >7 hours of stratospheric exposure (Liu J et al., 2022). Lichen exposure experiments using genetic amplification have determined that these microorganisms are more sensitive to UV radiation than to vacuum environments (de la Torre Noetzel et al., 2020). Kombucha microbial communities have been found to be resilient to environmental changes in space and Mars-like conditions (Góes-Neto et al., 2021). Additionally, no significant effects were observed on cultured bacterial strains when they were exposed to Mars-like gravity (Santomartino et al., 2020). Bacillus subtilis was able to sporulate and survive in Earth's upper atmosphere for 6 years (Horneck et al., 2010). Genomes of many bacterial and fungal species like Acinetobacter, Kocuria, Methylobacterium, Micrococcus, Pseudoclavibacter, Pseudomonas, Staphylococcus, Penicillium, and Aspergillus were isolated from the International Space Station using cell culture techniques (Blachowicz et al., 2021; Simpson et al., 2021).

Mars rovers have been equipped with instruments capable of collecting data of the following sorts that are useful in the detection of evidence of life: X-ray spectroscopy, reflectance and transmission spectroscopy, laser and Raman spectroscopy, mass spectrometry, electrochemistry, and imaging (Table 3). More recently, a UVRR spectrometer was loaded on the Perseverance rover (Manoharan et al., 1990). This device is able to detect evidence of microbial endospores, protoplasts, genetic materials, DNA secondary structures, aromatic amino acids, peptide conformations, and changes in oxidation state (Manoharan et al., 1990). ExoMars will carry the Mars organic molecule analyzer (MOMA), a versatile mass spectrometer, for comprehensive studies of carbonaceous molecules even at extremely low concentrations (Brinckerhoff et al., 2013; Arevalo et al., 2015). MOMA can deeply scrutinize molecular structures and species, such as monomers or chains of nucleic acids, amino acids, biolipids, and detect the chirality of sugars and amino acids. To date, however, circular dichroism spectroscopy, cell culture techniques, and nucleic acid sequencing have not been employed significantly in the Mars settings.

4. Discussion and Outlook

Missions to Mars continue to make progress in astrophysics and astrochemistry. Studies of the planet's habitability for Earth life forms, and studies to determine whether indigenous Martian life is present or has ever been present, are more and more imminent. Differentiating abiotic and biological signals is the most important and difficult step in Martian life detection. The difficulties come mainly from the requirement that all possibilities of abiotic origin must be ruled out if a finding is to be accepted as a true biosignature (Green et al., 2021). To exclude false positive findings as much as possible, multiple analytical techniques must be employed to confirm any tentative positive conclusion. In this review article, we have summarized and classified representative terrestrial Mars analog environments based on their similarities to the characteristics of different Mars periods; we have then associated with each environment the appropriate detection techniques that are currently available, and their associated instruments capable of being carried by spacecraft and possibly mounted on rovers to detect potential biosignatures on or near Mars. Moreover, to avoid overlooking any concealed biogenic fingerprints, we have suggested that the raw data or spectra themselves determined by instruments can be regarded as biosignatures. Each single point or measurement on a spectral curve (even that of a visible spectrum) more or less implies the physical and chemical properties of the measured material that can potentially be differentiated by inspecting relevant abiotic or biological effects (Li XL et al., 2007; Kaltenegger et al., 2010). We urge great care in the noise filtering step when future studies interpret spectral data.

Representative Mars analogs were grouped to match Martian environments of different geological times and locations. Numerous astrobiological studies have been conducted to address the identification of potential biosignatures and *in situ* rover detection methods suitable to each. Older Noachian and Hesperian terrains in the Southern Hemisphere of Mars appear to be promising targets for early Martian life detection (Golombek et al., 2003; Mittelholz et al., 2018); autonomous robotic tests performed in respective Mars analog environments have demonstrated that deployment on Mars is now feasible.

The remaining challenge is the landing issue: parachutes of Mars landers need enough atmospheric friction or landing distance to reach the surface without damage to instrument packages. The thin atmosphere of Mars makes this much more difficult than on Earth, especially in the planet's Southern Hemisphere, with its significantly higher average altitude than in the Northern Hemisphere (Cruz and Chadwick, 2000; Golombek et al., 2003). Resolution of this technical issue will enable the search for biosignature in the promising Southern Hemisphere, which will profoundly advance our knowledge of the habitability of early, wetter Mars. Other ideal Martian targets for habitability assessment and possible life detection include deep subsurfaces, caves, and subglacial water-ice reservoirs that are shielded away from surface sterilizing conditions (Cabrol, 2018). Drilling systems will undoubtedly be important as they are essential if the analytical instruments are to be exposed to subsurface targets of interest.

Based on previous robotic rover explorations in these environments, it now appears feasible to look seriously for direct evidence of life on Mars. However, due to the engineering and spatial restrictions of portable rover payloads, *in situ* Mars life detection will be challenging. Developed by collaboration among teams from ESA-Roscosmos and NASA, the rover Rosalind Franklin in the ExoMars project will be equipped with multiple, versatile, state-of-the-art instruments (i.e., the imagers PanCam and CLUPI, the reflectance spectrometer MicrOmega and ISEM, the Raman spectrometer RLS, the mass spectrometer MOMA, and even a subsurface IR-incorporated 2-m core drill unit) (Rull et al., 2022), whose data can be coupled with that from the Trace Gas Orbiter pre-launched in 2016 (Gibney, 2016). Therefore, the Rosalind Franklin possesses a high potential for detecting an unprecedented volume of first-hand data collected by various powerful devices. In the future, additional useful instruments (e.g., fluorescence microscopes, circular dichroism spectrometers, FTIR, trace gas sensors, μ Raman, *in situ* sequencers, cell culture systems, and redox indicators) can be designed for inclusion as portable payloads on future Mars rovers to detect life signals more comprehensively. Promising life-detection techniques that remain unmountable on rovers, or for other reasons are insufficiently well-developed to be deployed on Mars, can nevertheless be useful in laboratories on Earth when materials collected on Mars become available for analysis via sample-return projects.

Herein, we have proposed a strategic framework for biosignature detection on Mars (Figure 2). To plan a life detection scheme more purposefully and deliberately, we suggest that astrobiologists should first take the specific geological period and geological/ geochemical features of chosen landing sites into consideration (Westall et al., 2015). Then, based on this comprehensive information, one or more candidate terrestrial Mars analogs can be selected and investigated to understand the parallel environmental and possible biological conditions in the chosen landing sites on Mars. Next, assumptions can be made regarding whether life there is likely to be absent, dead, dormant, or active on a small/ large scale, or whether the environment may potentially be in a prebiotic stage.

We suggest that multiple research teams be encouraged to propose, independently, different possible target biosignatures and instrument-based detection approaches (Green et al., 2021). Finally, instrument sets that are proved to be promising and practical in trials on Earth can be deployed in actual future Martian life detection missions. If and when putative biosignature clusters that cannot be interpreted plausibly as the consequence of any known abiotic mechanisms have been detected, by multiple techniques, the same material or spot should be investigated further, at different temporal points within a relatively long duration, to verify and consolidate the suppositions by confirming that the evidence is repeatable. If sample-return missions are achieved successfully, laboratory-based techniques capable of making additional, complementary life-assessments will make these conclusions even more persuasive.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

J. X. Shen and W. Lin initiated the manuscript, designed the content, and wrote the manuscript. All authors reviewed and edited the manuscript before submission.

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Figure 2. Proposed framework for instrumental detection strategy of Martian biosignatures based on geological period and physicochemical features of targeted Martian sites.

List of Abbreviations

AO, acridine orange CNSA, China National Space Administration DAME, Drilling Automation for Mars Exploration EA, elemental analysis EBSD, electron backscatter diffraction EDS, energy dispersive X-ray spectroscopy EPMA, electron probe microanalyzer ESA, European Space Agency FIDO, Field Integrated Design and Operations FTIR, Fourier transform infrared GC, gas chromatograph HMPRS, Haughton-Mars Project Research Station HSDP, Hawaii Scientific Drilling Project IC, ion chromatography IR, infrared IRMS, isotope ratio mass spectrometry ISRO, Indian Space Research Organisation JAXA, Japan Aerospace Exploration Agency LC, liquid chromatography LIBS, laser-induced breakdown spectroscopy LIF, Laser-induced fluorescence MARTE, Mars Astrobiology Rio Tinto Experiment MOMA, Mars organic molecule analyzer µRaman, micro-Raman MS, mass spectrometry MSL, Mars Science Laboratory NASA, National Aeronautics and Space Administration NRVS, nuclear resonance vibrational spectrometer ORP, oxidation reduction potential PISCES, Pacific International Space Center for Exploration Systems SEM, scanning electron microscopy SIMS, secondary ion mass spectrometry SWIR, short-wave infrared UAE, United Arab Emirates UV, ultraviolet UVRR, ultraviolet resonance Raman spectroscopy VNIR, visible-near infrared WDS, wavelength dispersive spectroscopy XANES, X-ray absorption near-edge structure XRD, X-ray diffractometry XRF, X-ray fluorescence

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