Subsurface microbial observatories to investigate the deep ocean crust biosphere – development, testing, and future

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Abstract

Scientific ocean drilling has historically yielded some of the most transformative advances in the Earth sciences, cross-cutting many of its disciplines, and providing fundamental advances to our knowledge of how the Earth works. Today, ocean drilling is poised to offer these same transformative advances to disciplines within the life sciences, and provide insight into how life operates and interacts with Earth processes at and below the seafloor. To date, many exciting discoveries have been made about the nature of the deep microbial biosphere in marine sediments. In comparison, there is relatively little information about the nature, extent, and activity of microorganisms living in the volcanic oceanic crust. Because of the size and hydrodynamics of this potential biome, crustal life may have profound influence on global chemical cycles and, as a consequence, the physical and chemical evolution of the crust and ocean. Hence, it is imperative that the scientific community develops a more complete understanding of life in ocean crust. To do this, researchers must develop the appropriate tools for studying this unique habitat, and recent engineering and methodological advancements make now a particularly opportunistic time to do so. Subseafloor borehole observatories (CORKs) can help to provide representative samples of crustal fluids and microbiological samples, reducing the extent of contamination associated with drilling, coring, and other operations. Here, we provide an update on current microbial sampling and experiments using CORK technology and highlight future developments that will facilitate fundamental breakthroughs in understanding microbial life in the oceanic crust.

Background

Exciting discoveries made during the last 20 years are revolutionizing our understanding of microbial life in deep marine sediments (for example, 5, 8, 12), but comparatively little is known about the nature of microorganisms within the volcanic oceanic crust. Large areas of volcanic crust are exposed to bottom seawater and serve as the conduits of exchange for fluids, heat, solutes, and biological material between the oceans and the lithosphere. The crust in some areas is buried by thick layers of sediment and is now known to be hydrologically and biogeochemically active, even out to ages >100 Ma. The rate at which water flows to and from the ocean crust rivals the flow of all the world's rivers into the ocean, and most of this flow occurs at low to moderate temperatures (0-40^{o}C) on ridge flanks, at distance from the magmatic influence of seafloor spreading. The upper oceanic crust is the most voluminous, continuous aquifer system on the planet, yet we know little about what kinds of life this habitat may harbor, what strategies microorganisms employ to generate energy and acquire carbon, or how similar/different these microorganisms are to the deep ocean, deep sedimentary, and continental realms. It is essential that the scientific community explore the ocean crustal biosphere, to develop a more complete understanding of the function, activity, size, and consequence of this biosphere in order to elucidate how microbial activity in the ocean crust may influence global chemical cycles and crustal properties. Scientific ocean drilling will play a critical role in these studies.

There are numerous challenges that must be met if we are to develop a fundamental, process-oriented understanding of the oceanic crustal biosphere. One profound challenge is avoiding the confounding influence of contamination during sampling, drilling, and experiments. Microbial communities are likely enriched where the crust is most permeable – the same areas that are the most susceptible to contamination by drilling, due to the introduction of shallow ocean water (used as drilling fluid), pipe lubricants, mud, and other materials. One promising approach to overcome potential microbial contamination during drilling is through the use of subsurface observatory systems, known as CORKs (1,4), where a borehole is cased and depths of interest are isolated and sealed, allowing the system to recover (thermally, chemically, microbiologically). Pioneer efforts in observatory systems were initiated to address questions primarily concerning geophysics, hydrogeology, and chemistry, and did not prioritize limiting potential sources of contamination for microbiological studies; however, in recent years, microbiologists have begun to collaborate on these efforts, focusing on design and methodology to reduce contamination (Fig. 1A; 3, 6,9).

Description of subsurface microbial observatory technology

Tools currently available for CORK-associated microbial observatory experiments can be broken down into two categories: those that are deployed down-hole ("subsurface") within the CORK casing, and those that are deployed at the seafloor and connected to the horizon of interest via pumping of fluids through umbilicals. Tools within these categories are not mutually exclusive. In fact, redundancy between seafloor and subsurface sampling and experimental units allows for a higher confidence of capturing representative samples for targeted questions. Each category has its own benefits and disadvantages, highlighted below.

The first generation of downhole observatory technology consisted of subsurface temperature and pressure loggers and osmotically-driven fluid samplers ("OsmoSamplers") which cumulatively collect a continuous record of temperature, pressure, and composition of the fluid within CORKed boreholes (for example, 3, 16, 17). Some of these samplers can be connected at the seafloor to tubing that extends to depth within a borehole, whereas others are deployed deep within a borehole and must be retrieved in order for samples to be recovered and analyzed. A second generation of downhole devices consists of microbial colonization experiments of polished mineral substrates deployed in contact with the borehole fluids (6), and microBiological Osmo Samplers (BOSS) connected to the same style of pumps used in the OsmoSamplers, which collect continuous small volume samples of borehole fluids for microbiological analysis (7). Comprising the next generation of downhole colonization experiments are Flow-through Osmo Colonization Systems (FLOCS, Fig. 1B,C), designed to encourage the growth of *in situ* microorganisms onto mineral and solid substrates that mimic the surrounding habitat (11). All downhole technology is limited by the lateral dimension of the experimental environment (i.e. all instruments must fit within the innermost borehole casing, which is typically on the order of 9 cm diameter). Downhole instruments also must provide necessary power for the duration of the deployment, which can last 4-5 years.

In contrast, CORK instruments deployed at the seafloor have fewer dimensional limitations, can be deployed with large battery packs to accommodate instruments with higher power consumption demands, allow for large volumes of water to be sampled, and are easier to service than downhole instrumentation. However, seafloor sampling instruments require umbilical lines for fluid delivery through the CORK from the depth horizon of interest. Pumping of fluids through the umbilicals is usually required, even in overpressurized holes, in order to obtain the large volume samples and/or high flow rates such as typically required for microbiological studies (flow rates of up to 5 L/min are possible using 1.25 cm diameter fluid delivery lines). Current seafloor microbial observatory technology includes several components (Fig. 1D; Cowen et al., in prep.): (*i*) a robust instrument sled capable of travel between surface ship and seafloor as a free vehicle, (*ii*) a pumping manifold for simultaneous connection to multiple CORK fluid delivery lines and multiple samplers, (*iii*) various downstream large and small volume samplers for collecting filtered and unfiltered samples for microbiological analysis, and (*iv*) an In Situ Electrochemical Analyzer (ISEA) capable of logging automated voltammetric measurements of redox-sensitive chemical species (e.g. 10). Existing seafloor observatory instrument sleds have been deployed for short-term (hours to a few days), with long-term (1 yr) deployments currently being planned. To guide remote sampling, future sleds will also be interfaced with technology under development for remote analyses of fluid composition. These technologies include additional electrochemical and optical sensors being interface with the ISEA, and the coupled fluid degassing system (SeGass) and In Situ Mass Spectrometer (ISMS) for analyzing concentrations of dissolved gases likely influenced by microbial processes, such as methane and carbon dioxide (15).

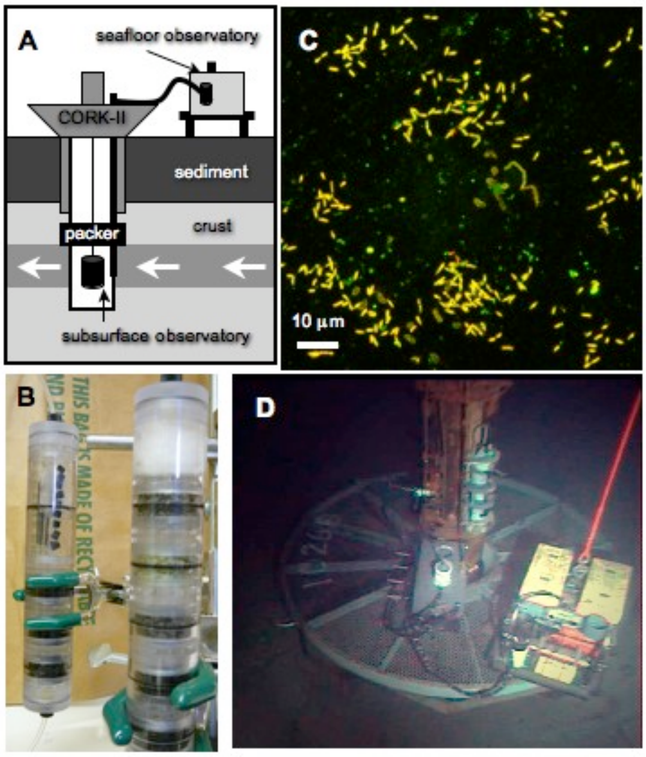

Figure 1. Schematic illustrating the deployment of subsurface and seafloor observatories in association with a CORK borehole targeting fluid flow in permeable section of the ocean crust (A), photograph of FLOCS chambers packed with mineral substrates (B), photomicrograph of cells that have colonized a FLOCS experiment (C), and photograph of a seafloor observatory instrument sled (D).

Needed technology development

The continuing adaptation of technologies from other disciplines will advance capabilities to observe and sample the subseafloor crustal biosphere. Technologies that are suitable for long term deployment, with ultra-low power consumptions and minimal impact by biofouling, are ideal for crustal biosphere observatories. More instrumentation for making remote measurements of downhole conditions is also desired. This includes designing downhole electrochemical and mass spectrometer analyzers, for measuring changes in fluid and gas compositions, and also developing new ways to measure rates of chemical reactions *in situ.* For example, a protoype downhole sampler for manipulative (e.g. tracer) experiments is nearly ready for field trials (Cowen and Taylor, in prep). Another promising adaptation would be instrumentation for measuring deep UV fluorescence downhole, permitting the detection of the native fluorescence of microbial cells without the use of stains or dyes or interference from auto-fluorescent mineral particles. Deep UV fluorescence technology has been in development as a method for life detection in astrobiology fields for several years (2, 14); adaptation of this technology for coupling with wireline logging tools and subsurface observatories would assist in identifying where native populations are present, and for resolving microbial populations that develop within borehole observatories. The Environmental Sample Processor (ESP), a field-deployable hybridization assay instrument that can detect the presence of certain microbial groups based on genetic markers (13), could also be adapted for connection to seafloor observatory systems to monitor the presence and relative abundance of target microbial groups. Deep biosphere research would also benefit from the development of tools that would allow for colonization experiments to be collected under *in situ* temperatures and pressures, for further manipulation in laboratory-based studies.

Future observatory experiments will also benefit from the utilization of components that are compatible with objectives in multiple disciplines (microbiology, hydrogeology, chemistry, etc.). For example, CORK boreholes are commonly comprised of low alloy steel casing, which is corrosive and can potentially compromise microbiology-driven experiments. Current research has identified that usage of alternative casing materials, such as fiberglass and coated steel pipe, may be more suitable for microbiological objectives (11). Fluid delivery lines in recent CORK deployments use PVDF-Titanium or 316 stainless steel (6). New fluid delivery lines will be constructed of either PVDF or another fluoropolymer, highly inert and chemically/thermally resistant materials that should not compromise fluid chemistry or microbiology samples.

In tandem with improving observatory capabilities for microbiological experiments, drilling tools are also needed that can recover relatively pristine, microbiologically-uncontaminated crust samples. Possession of higher quality crustal drill core samples from the areas with CORK experiments will provide a better baseline for monitoring the chemical, physical and microbiological conditions of the boreholes.

Realities of subsurface microbial observatory programs

Deploying CORK-based observatories requires significant advanced planning beyond traditional scientific ocean drilling programs. Extended fundraising efforts independent of access to scientific ocean drilling programs are needed; sufficient lead time to identify appropriate technologies and vendors, and to design, prototype, and test experimental systems is crucial. Advanced engineering efforts are necessary to design CORK platforms that maintain a complete and continuous seal against the surrounding environment to isolate individual depths in the crust and prevent contamination. Seafloor and downhole instrumentation requires periodic servicing by submersible or remotely-operated vehicle, and this entails careful operational scheduling of drilling, oceanographic, and other research assets and personnel. Following drilling and observatory installation operations, it may take months to years for a borehole to return to natural conditions; however, such disturbance-rebound sequences can provide extremely valuable opportunities for time-series microbiological, geochemical and ecological observations. A great challenge to achieving fundamental breakthroughs in ocean crustal microbiology during scientific ocean drilling is the time required to plan, secure support, and complete experiments, especially in light of the limited time that is typically made available when annual drilling schedules are set. Microbiological experiments using CORK observatories require time for design, fundraising, and instrument construction well beyond the <1 year planning cycle for IODP. New financial and planning models for observatory systems science in ocean drilling are required for the promise of transformative advances to be realized in the next phase of ocean drilling.

Conclusions

Subseafloor microbiology is a relatively new discipline within the scientific ocean drilling community. Recent experience shows that we can resolve critical questions involving the subseafloor biosphere if we plan carefully, embrace the latest technology, and allow enough time for proper planning and implementation. Subseafloor microbiology is changing our view of how life formed and developed throughout Earth history, and may provide insights as to what kinds of life may be found one day on other planetary bodies. Much as plate tectonic theory revolutionized Earth Science, studies of subseafloor microbiology have a profound impact on fields such as developmental biology and biogeochemistry. New experiments can take advantage of existing boreholes and observatory systems, and by leveraging these systems with collaborators in complementary disciplines, we will make scientific ocean drilling stronger as we pursue this exciting topic of research. Making major advances will require a dedicated effort to schedule, plan, prototype, deploy, and service microbial observatory systems, which necessitates making a long-term commitment to these kinds of experiments and to developing the infrastructural and financial models that are fundamentally critical for their success.

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