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Artificial Microbial Arenas: Materials for Observing and Manipulating Microbial Consortia

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From the smallest ecological niche to global scale, communities of microbial life present a major factor in system regulation and stability. As long as laboratory studies remain restricted to single or few species assemblies, however, very little is known about the interaction patterns and exogenous factors controlling the dynamics of natural microbial communities. In combination with microfluidic technologies, progress in the manufacture of functional and stimuli-responsive materials makes artificial microbial arenas accessible. As habitats for natural or multispecies synthetic consortia, they are expected to not only enable detailed investigations, but also the training and the directed evolution of microbial communities in states of balance and disturbance, or under the effects of modulated stimuli and spontaneous response triggers. Here, a perspective on how materials research will play an essential role in generating answers to the most pertinent questions of microbial engineering is presented, and the concept of adaptive microbial arenas and possibilities for their construction from particulate microniches to 3D habitats is introduced. Materials as active and tunable components at the interface of living and nonliving matter offer exciting opportunities in this field. Beyond forming the physical horizon for microbial cultivates, they will enable dedicated intervention, training, and observation of microbial consortia.

1. Introduction

Here, we aim to highlight opportunities for materials research in providing new tools and artificial habitats for the study of complex microbial consortia. We introduce general material concepts and methods to fabricate artificial microbial arenas for the systematic investigation and manipulation of microbial interactions.

Communal living is a hallmark of life on Earth. No matter how small an interaction partner is, interactions between organisms have dramatic influences on most biological niches, if not on the entire world.[1] Networks of interacting organisms with intricate spatial and temporal structures are critical in the maintenance of health for plants, animals, and humans, and are also prerequisites for functioning ecosystems,[2,3] stable climates,[4] and sustainable agriculture.[5] At the heart of these complex biosystems is the microverse,

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which we define as a collection of functionally interacting and spatially coexisting microbial consortia. A microbial consortium is a collection or association of microorganisms. Within such consortia, numerous layers of interaction are governed by chemical, physical, and biological mediators that then control the composition and structure of the entire community.

Over the past decades, research into microbial dynamics has mainly focused on individual species or species in bilateral or tripartite interactions.[6] However, such experiments do not reflect the structural and functional diversity, or the interaction patterns that are pertinent to the true ecological context.[7] Under standardized laboratory conditions using pure culture studies, many genes encoding for biosynthetic pathways remain silent,[8] suggesting the importance of complex multispecies interactions.[9] Only more detailed investigations of multispecies interaction and metabolite proliferation in the microbial consortium within a particular host[10] (e.g., the human gut microbiome) or within a particular environment[11] (e.g., a water or soil microbiome) provide insight with immediate implications on fields such as medicine, biotechnology, ecology, or environmental science.

Microbial consortia occur in structured and unstructured environments, i.e., environments with or without a physical barrier or containment (**Figure 1**). An example of the latter is the microbial community of open water oceans. Here, microalgae, bacteria, and viruses form networks with pronounced and—to a certain degree—predictable seasonal variations.[12] In such physically unstructured environments, gradients of natural products released by the microbial players can act as mediators for niche formation.[13] In more structured or physically confined environments such as in biofilms on surfaces, the concerted action of complex microbial populations is often more obvious. It is a startling fact that microorganisms colonize the skin and internal surfaces, gut and colon of insects, vertebrates, and humans.[14] The number of microorganisms in our bodies is of the same order of magnitude as the number of human cells.[15] There is evidence that the microbial species diversity and their community structure and functions influence not only digestion, but also immunity, inflammation, development of cancer, and even psychosocial traits of their hosts.[16,17] In analogy, infections often involve biofilm formation and colonization by more than a single pathogenic microbe. Such mixed infections are highly difficult to treat and pose a serious threat to the health of humans, animals, and plants.[18] Thus, health and performance of plants, animals, and humans critically depend on their associated microbiomes.[19,20] The pertinent networks of organisms and their microbiomes can be considered as "superorganisms," i.e., organisms formed by a large number of individuals.[21] Superorganisms can only survive when kept in a certain state of balance, and their metabolism represents an amalgamation of microbial and host attributes (from a materials science perspective, the creation of artificial habitats that mimic micro- to nanostructured environments appears to be straightforward, whereas the conditions that occur in unstructured consortia or parts thereof pose a greater challenge).

Acknowledging the fundamental role of interspecies interaction in regulating microbial consortia, microbiomes, and, in consequence, the microverse, present research is focusing on four fundamental questions:[22] 1) Which species are present in a given consortium, in time and space? 2) What is the specific

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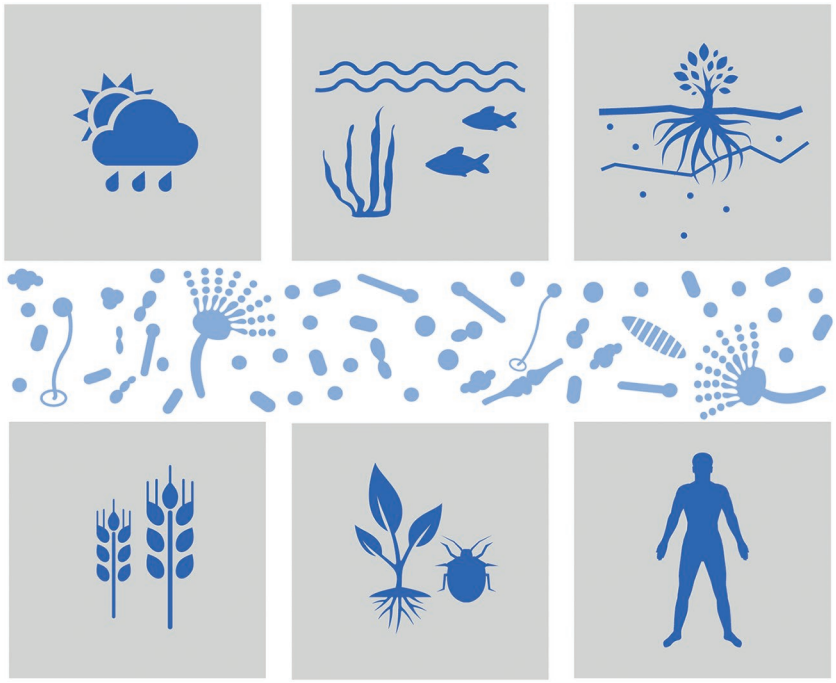

Figure 1. Occurrence of microbial consortia in physically structured and unstructured environments, from air, water and soil to plants, insects, and the human body.

role and function of each species? 3) How do consortia interact on a single-cell level? 4) Can meaningful predictions as to the composition and the dynamics of a consortium be made?

With these overarching questions in mind, research into new materials and material fabrication processes aims at developing devices that enable the stable cocultivation of microbial consortia, from binary to multispecies or even natural assemblages (**Figure 2**). Combining modern material fabrication techniques, functional surfaces and stimuli-responsive materials, consortium stabilization, and directed evolution can be supported through modulated stimuli or through initiating spontaneous response triggers. We expect that using such habitats in the study of microbial interaction patterns will provide new opportunities for the understanding of control triggers and fundamental interaction parameters, conditions for generating and disturbing microbial balance, the generation of synthetic and trained microbial consortia, and the conception of living material–based on higher-order community structuring. In return, we anticipate new momentum for the generation of materials allowing to control the interaction with living matter, for example, through antibacterial/antimicrobial functions and nonadhesive or antifouling surfaces.

2. Microbial Balance and Materials for Mediating Adapted Stimuli

In order to understand functional dependencies and strategies for predicting the evolution of a given microbial consortium, accurate knowledge of the communal dynamics, balancing parameters, and factors of disturbance is required. While some fundamental data can certainly be collected in natural environments, in-depth laboratory experimentation is indispensable in order to elucidate cause–effect relationships; thus, the need for advanced artificial habitats that can mimic the dynamics of structured as well as unstructured environments and provide readout functions. On

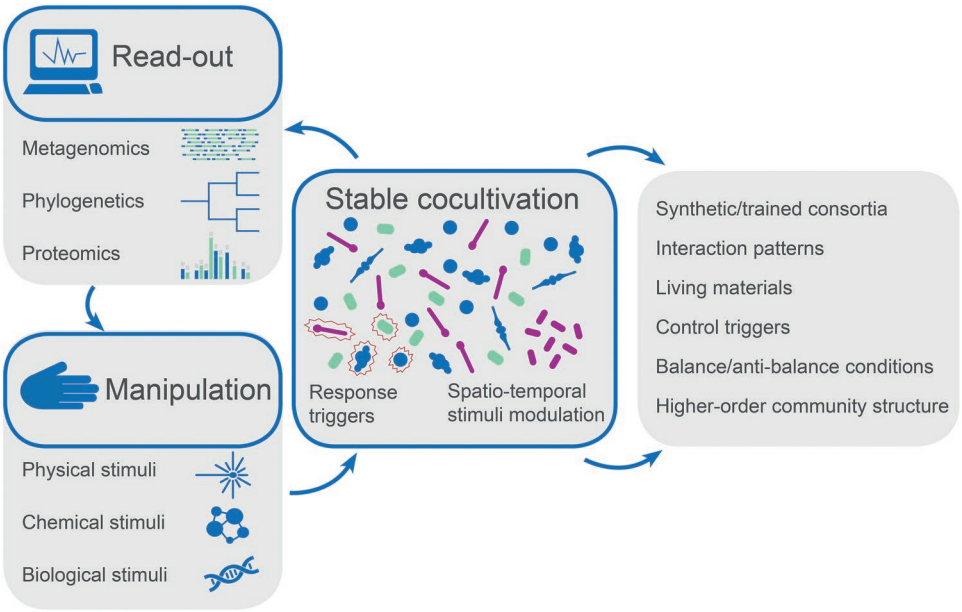

Figure 2. Artificial microbial arenas for the cocultivation of binary and multispecies microbial consortia.

this area, we expect significant opportunities for the conception of new materials and associated strategies of material fabrication and integration.

In natural microbial consortia, the general issues of balance and persistence have been subjects of longstanding debates,[23] primarily because microbial consortia exhibit highly nonequilibrium dynamics.[24] Subject to a broad range of parameters, a given consortium may attain distinct states of balance,[25] but also evolve through competitive exclusion,[26] within limit cycles[27,28] or in deterministic chaos.[29,30] A key factor in the attribution of any of these states is the timescale of observation: rapid limit cycles may appear as metastates within a stable basin, and what appears as a chaotic evolution may be part of one or more overlapping limit cycles with slow kinetics. This will put stringent limitations on future material design, depending on the intended application. For example, materials used for the construction of artificial habitats need to provide buffering or responsive functions on various timescales, for which detailed knowledge must be generated on the kinetics of stimulus transmission and response capacity at material interfaces.

According to the current viewpoint,[23] there are two aspects of balance or stability to be emphasized, i.e., stability in numbers (constancy) and stability in the qualitative presence of each community species (persistence). Because microbial consortia are confronted with continuously varying external and internal stimuli, the latter appears to be the more suitable characteristic to define a state of stability.[31,32] This leads to the primary criterion of stability, whereby for a stable system, a quasi-isostatic situation or limit cycle exists in which the system remains or to which it returns when disturbed by an external stimulus. To stay with the above example, a stimulus-responsive component of the habitat itself can be employed to facilitate balance by compensating any de-balancing factor or to gradually guide the consortium toward a target state (denoted as *training* in Figure 2). As illustrated in **Figure 3**, this accounts for intrabasin dynamics, which may break constancy but not persistence.

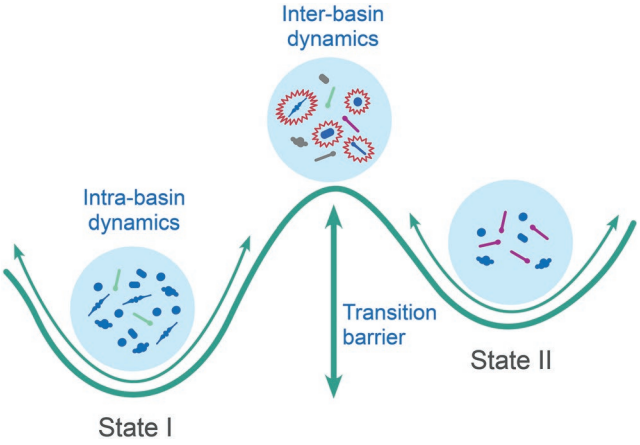

Figure 3. Microbial consortia with alternate states of balance. Stable consortia are determined by intrabasin dynamics (consortium resilience) separated through a transition barrier in the energy landscape representation. Transient states (interbasin dynamics) are subject to system disturbance and imbalance, for example, caused by chemical, physical, or biological stimuli such as mediated through responsive materials.

Numerous parameters contribute to the establishment of intrabasin balance, its disturbance, and the occurrence of multiple stable states. These include, e.g., population density, nutrient availability and the interaction strength of food webs,[33] the tragedy of commons,[34] predator–prey interaction and metabolic flexibility of predators,[35] metabolic cooperation,[36,37] Allee effects,[38] and catastrophic disturbance.[39] Elaborating on Holling's initial notion,[40] the term "resilience" has been adopted from network theory to describe a stable community's resistance to breakdown.[41] In a natural environment, pressure on resilience arises from exogenous factors such as temperature, atmosphere or the presence of pollutants and toxins, loss in trophic depth, reduction of diversity, or the loss of functional groups.[42] We recommend replicating such effects using a material-based approach that endeavors to manipulate resilience by adapting to a suitable stimulus. This involves the height of the transition barrier in Figure 3, the width of the basin and its capacity for intrabasin dynamics, and the interaction with alternative states of stability (backward reactions); all these parameters are affected by the local surface properties of the material from which the (artificial) habitat is constructed.

The initial motivation to devise materials that enable responsive habitats arises from the need for stable cocultivation of microorganisms, mimicking natural microbial consortia. Little is known about stability and resilience of microbial consortia within natural (structured or unstructured) environments. For example, microbial plankton communities of open ocean water undergo rapid switches between alternative states dependent on seasonality and associated fluctuations of nutrients.[2,12] However, similar alternate states are not known for other environments, e.g., groundwater microbiomes due to remarkably stable physicochemical conditions over the whole year.[43] Dark groundwater lacks the input of photosynthetically derived organic carbon from algae, which limits the productivity.[44] Such oligotrophic conditions lead to microbes with streamlined genomes that benefit from reduced reproduction cost, but face challenges due to the loss of metabolic functions.[45] The coevolution of microbial consortia with their respective environment suggests as-of-yet unknown differences in their response to perturbations, their level of resilience, and stability. Thus, to unravel the intricate interaction mechanisms of organisms within various environments, new experimental approaches are required.

High-throughput screening (HTS) and multivariate statistical analyses have become common tools in the study of microbial communities.[46] Despite high expectations for the future, however, even for state-of-the-art HTS, it appears that only the most general features of communal dynamics are presently assessable.[47] Once again, artificial environments in which certain factors such as the surrounding conditions or the local presence of chemical mediators can be precisely tailored in space and time offer an intriguing way to address this issue. Such habitats—now denoted "microbial arenas"—allow for the study of the response of communities to well-defined stimuli. A reduction of complexity is achieved through controlling the accuracy and the reproducibility of manipulation. This subsequently enables system parametrization and elucidation of the overarching patterns that occur in the dynamics of model consortia.

3. Artificial Microbial Arenas

In nature, microbial consortia evolve in an environment of continuously fluctuating properties.[32] Variations occur over space and time for nutrients, extracellular signals, and physical constraints. Conventional incubation experiments test the effect of specific growth and stress conditions on pure cultures of microbial species by either monitoring stress responses of the total microbial community or on single cell level of the same species. However, the behavior of pure, gnotobiotic cultures does not reveal the response of a species within a naturally occurring community. On the other extreme, harnessing the full complexity of a natural microbiome under laboratory conditions and in a viable time series will demand experimental and computational power to an extent which is presently not available.[48] Laboratory experimentation must therefore be conducted in synthetic arenas with reduced complexity.[48,49]

Microbial arenas are artificial, spatially confined habitats for microbial consortia (**Figure 4**). Their functionality arises from the materials used for construction. As a fundamental characteristic, they exhibit a microbiome with tailored complexity, evolving from a natural model or synthetic consortium. Particularly in the latter case, interaction among simplified (binary or few species) assemblages can be studied in environmental context, applying isostatic conditions or using response triggers. In this way, diversity and species compositions can be directed toward desired ratios and along pre-defined gradients.

Materials used for the construction of arenas act in both ways passively and actively. They provide the support structure and the physical horizon of the community, but also additional functionality for active control, manipulation and observation. Typical response triggers can be changes in local interface properties such as the surface pH-value, switchable transitions between hydrophobic and hydrophilic surface states, variations in surface roughness across various length scales, adjustable zeta potential (surface charge), the spatially and temporally controlled release of compounds (e.g., salts, small molecules, genetic material, etc.), or the local availability of light and electrical or magnetic fields. Using these parameters, complex states of coherence are generated at the interface between the living and the nonliving parts of the arena and are continuously transmitted into the contained consortium. In this way, dedicated states of balance and disturbance are generated not only for in-depth study, for example, of bacterial behavior,[50] but also for the exploitation of microbial consortia such as in *"living materials."*[51] Furthermore, trained consortia with distinct ecological functions[52] can be generated, or otherwise uncultivable microbiomes can be harnessed.[53,54]

The various use cases of artificial arenas require modular adaptation such as provided by modern material fabrication technologies. Then, combinations of reversibly switchable environmental conditions with local and global readouts may form a basis for integrating concepts of machine learning and artificial intelligence into studies of microbial interaction. For example, feedback-loops between external triggers and the response of a microbiome can be used to design autonomous experiments targeting specific community responses. Challenges for materials research lie in the exploration of materials featuring tailored buffering capacity and adaptive or responsive functions, and in the conception of fabrication technologies which enable arena fabrication in the desired combinations of materials, at varying dimensionality and length scale.

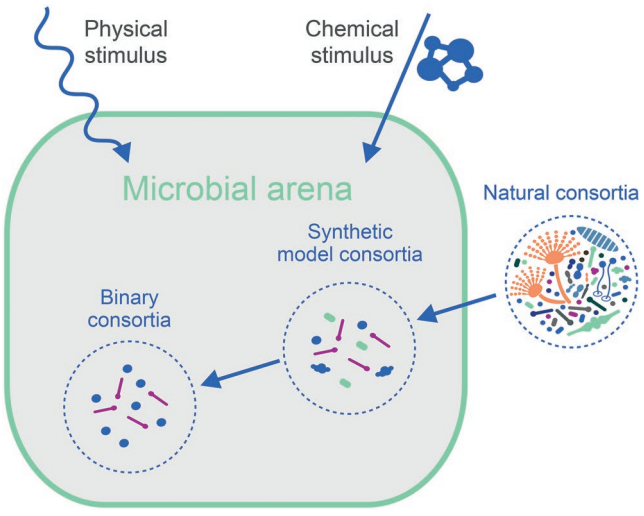

Figure 4. Harnessing microbial consortia through reduction of complexity. Microbial arenas enable the study, manipulation, and exploitation of synthetic model consortia and binary communities under external stimulation, with and without environmental exchange.

4. Arena Fabrication

4.1. Cultivation in Spatial Separation

The engineering of synthetic microbial consortia has been facilitated by rapid progress in microfluidic technologies,[55,56] primarily driven through the development of microbial biosensors.[57] Microfluidic reactors and microelectromechanical systems (MEMS,[58]) with one or more cells, channels, mixers, valves and membranes are fabricated *on chip* and/or arranged in multilayer systems through established fabrication technology from various materials.[59] This includes the fabrication of complex in vitro models with the objective to mimic in vivo interactions on organ level,[60] and multicell setups for the observation of natural consortia such as from the plant rhizosphere[61] or communities of bacterioplankton.[62] By using partially permeable shielding membranes, microbial consortia with incommensurate growth conditions can be spatially linked,[63] or depending on viewpoint, commensurate species can be separated[64] (**Figure 5**). Prominent examples of the former are given by advanced models for in vitro studies of the human gastrointestinal microbiome such as the HMI[65] or the HuMiX modules.[66] While membranes act to generate diffusion-controlled gradients in the presence of chemical compounds, convective gradients can also be established by using flow-based systems.[67] The overall strategy of such approaches is the tailoring of stability by spatial optimization:[68] through well-controlled separation of chemical reactions, yet maintaining spatial proximity and the ability to chemically communicate and exchange metabolites, community breakdown, for instance, caused by the dominance of a single or few species,

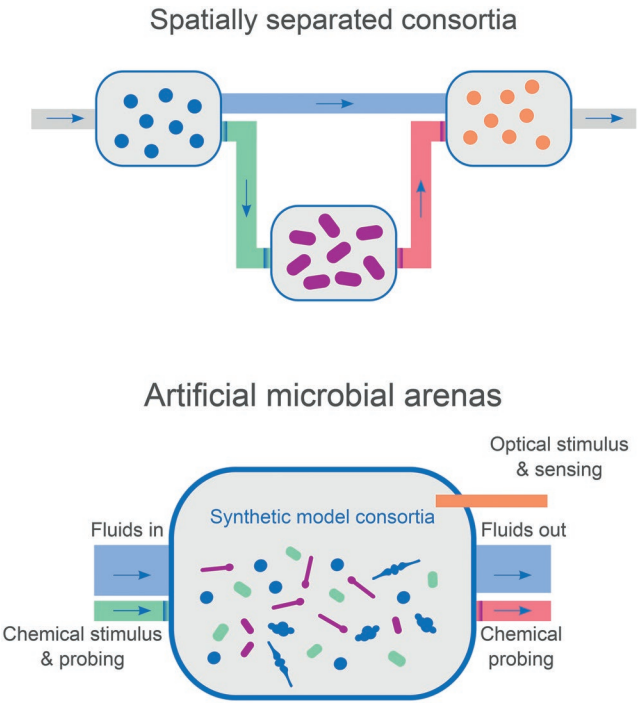

Figure 5. Physical separation in spatially linked consortia (top) and artificial microbial arenas for cocultivation (bottom). The top scheme was adapted under the terms of the CC-BY 4.0 Creative Commons license (https://creativecommons.org/licenses/by/4.0/).[63] Copyright 2017, The Authors, published by Frontiers Media SA.

is avoided. For example, laboratory cocultivation of syntrophic species typically requires an intricate balance of exogenous factors and nutrient supplements, or it may never achieve stability at all. Spatial separation has proven a useful tool to overcome this issue.[69–71]

On the other hand, cocultivation remains highly desirable for the reasons already mentioned above: only in complex cocultures or natural microbial consortia, overarching interaction patterns such as niche formation or species sampling, division of metabolic labor or spontaneous spatial and temporal organization can be observed.[7]

4.2. Arenas for Cocultivation

The design of microbial arenas for the cocultivation of complex microbial consortia benefits from material processing strategies which go beyond simple machining, 2D lithographic methods, hot embossing, direct forming and laminate assembly such as typically employed for planar microfluidic devices.[59,72] Most notably, the incorporation of secondary functionality on part of the material from which the arena is constructed enables the simulation of adaptive environmental conditions, and provides tools for the generation of exogenous stimuli, for sensing and for observation. For this, processing technologies are required which are capable of introducing organic, inorganic and hybrid materials with different mechanical properties, surface states and mesostructural density. These technologies also need to implement various chemical or physical functionalities, and equip structures with gradient properties, for example, gradients in the presence of a certain chemical substance. In addition, all these features have to be made available at spatial selectivity ranging from micrometers to a few nanometers, for device sizes which exceed several centimeters, and, potentially, for compatibility with classical microfluidic, inkjet and MEMS technology.

Table 1. General characteristics of single-cell cultivation and artificial microbial arenas with increasing complexity.

	Single-cell cultivation	Artificial microbial arenas			
		Particulate microniche	1D habitat	2D habitat	3D habitat
Cell number	1	$>10^2$	$>10^2$	$>10^3$	$>10^4$
Length scale	µm	µm	µm	µm–mm	µm–cm
Volume range	fL	pL–nL	pL–nL	nL–µL	nL–mL
Community homogeneity	n.a.	+++	++	+	–
Implementation of gradients	n.a.	–	+	++	+++
Ability for spatial separation	n.a.	—	–	+++	+++
Exchange reactions	Diffusive	Diffusive	Diffusive, convective	Diffusive, convective	Diffusive, convective
Readout by imaging	+++	++	+	+	–
Readout by sampling	n.a.	–	++	++	+++
Examples of design	Hydrogel nanodroplets, lithographic nanoniches, artificial vesicles	Soft/hard microbubbles and vesicles, mesoporous microparticles, particle surfaces, hydrogel composites	Microcapillaries, microstructured fiber, integrated microchannel reactors	Microstructured glass/plastic substrates, laminated substrates, thin-film membranes	Hydrogels, inorganic scaffolds, microporous glasses, polymeric scaffolds, mesoporous membranes
Examples of fabrication	Droplet microfluidics, nanolithography	Microfluidics, sol–gel chemistry	Fiber drawing, fiber spinning	Inkjet printing, micro/nanolithography, hot embossing, soft lithography, spin-coating	3D printing, gel casting, cryo-gelation, templating, self-assembly

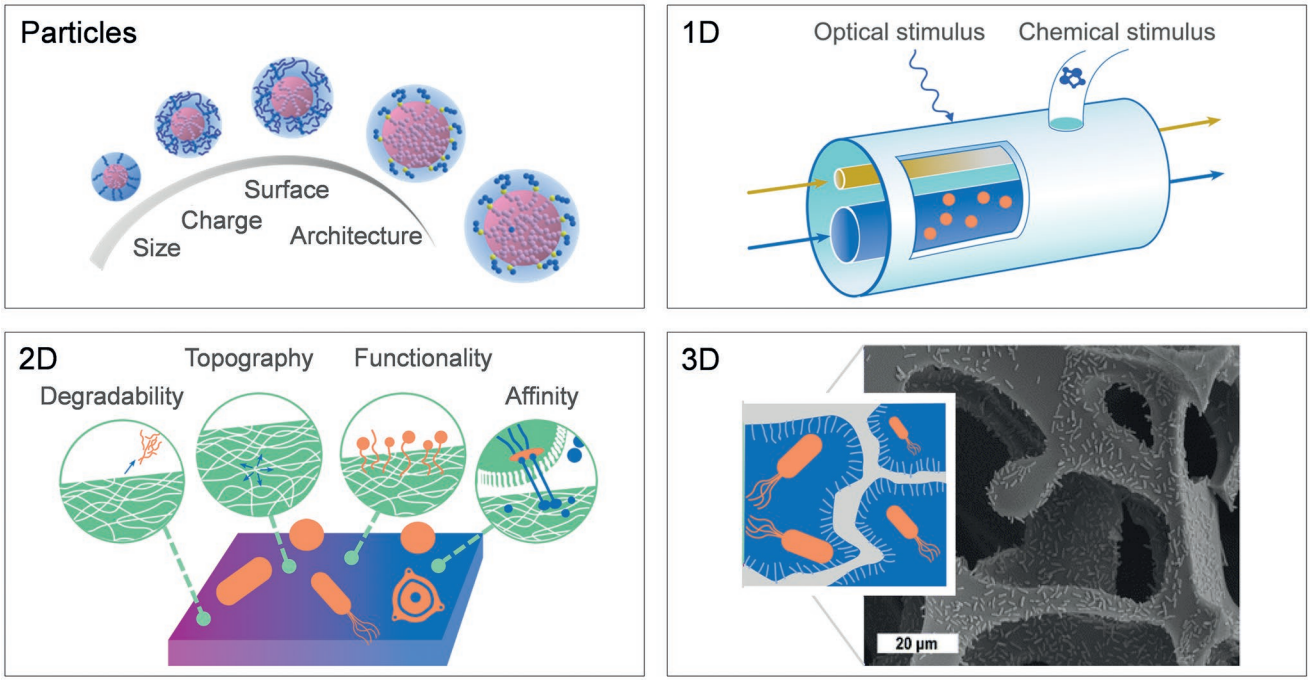

Figure 6. From functional particles to 3D scaffolds: microbial arenas with increasing dimensionality. Lower right: adapted with permission.[124] Copyright 2016, American Chemical Society.

Drawing an analogy to single-cell microfluidic engineering,[73] some primary characteristics of artificial microbial arenas are summarized in **Table 1**. According to arena geometry, we distinguish particulate microniches (smart release- and sensor particles or microcapsules which incorporate microbial consortia), 1D (hollow, geleous, or composite fiber), 2D (tailored surfaces with gradient or switchable properties), and 3D arenas (tailored and/or switchable environments in 3D scaffolds with tailored porosity) (**Figure 6**). Per se, the present concept does not involve 0D single-cell habitats. Differentiation of particulate microniches from 3D environments may be ambiguous; here we draw a line for particulate materials with a characteristic scale of <100 µm (e.g., vesicles, microbubbles) which do not exhibit a further internal mesostructure so as to distinguish from intentionally 3D-structured materials.

On each of the above dimensions, advanced processing techniques allow for the generation of pre-defined and reproducible gradients in chemical and physical properties, and, hence, the directed cultivation of microbial species.

Drawing on their compatibility with general microbiological processing, platform materials of interest involve the classical candidates from planar microfluidic technology, i.e., silicon, certain polymers, glasses, composites, and hydrogels.[59] Adding further functionality, these can be complemented by responsive and smart polymers,[74] nanostructured block copolymers,[75] metalorganic and other hybrid materials such as metal-organic frameworks[76] or hybrid mesoporous silica,[77] optically active metallic or nonmetallic particles, quantum dots and dyes,[78] functionalized colloidal or thin-layer nanozeolites,[79] or glasses with controlled nanoporosity.[80] Multidimensional structuring can involve nanolithography, laser-assisted additive manufacture, inkjet printing, imprinting and hot embossing, fiber and textile processing, and template-based techniques and self-organization.

Colonization of surfaces by microorganisms is a major issue, both in natural and artificial environments:[81,82] the majority of microbial life resides in the subsurface, in close association with geogenic surfaces.[83] Across multiple length scales, the surface properties of substrate materials determine the interactions with microorganisms or microbial consortia.[84] Modern materials chemistry offers several tools to structure artificial surfaces and interfaces for mimicking or complementing conditions which are found in nature. For example, there have been strong efforts to control biofilm formation through engineering surface chemistry, physical properties and topography,[85] often with the intension to prevent adhesion of microorganisms.[86] Moreover, the topography of a micropatterned surface on which a coculture biofilm grows has important implications for species colonization, growth, and persistence when exposed to antibiotic agents.[87] Further control can be achieved by installing different chemical functionalities, homogeneously spread across a surface of a certain dimensionality, combined in close proximity on a patchy surface, or by producing lateral surface gradients of polarity,[88] charge, stiffness, or microstructure.[89] Dedicated release properties can also be generated, where a specific chemical compound or metabolite is emitted from the surface in spatiotemporal control. This offers even further opportunities for the regulation of microbial communities, when suitable chemical mediators are involved. For example, stem cells in contact with materials are able to sense their properties, integrate cues via signal propagation and ultimately translate parallel signaling information into cell fate decisions.[90] Gradient or patchy surfaces on nanoparticles,[91] fiber,[92] flat substrates,[88,89] and in 3D structures are increasingly used to induce specificity or mimic a certain environment such as the extracellular matrix (ECM) for several purposes, e.g.,

in the fields of tissue engineering and regenerative medicine. Gradients can also be used to introduce a controlled degree of complexity into a biomaterial and, in turn, guide organism or cell behavior.[93] Even more direct structuring on the nanolevel can be realized applying approaches such as dip-pen lithography or AFM-based oxidation techniques.[94,95]

There are myriad technologies for fabricating particulate microniches. In the most straightforward way, solid particles of virtually any type can be used to mimic certain features of wild, particle-attached microbial communities, for example, those occurring in open waters.[96] Directed community assembly on synthetic microparticles has also been reported for the degradation of particulate organic matter in seawater.[97] In a more general consideration, there is now a broad variety of examples where microbial consortia have been immobilized on various kinds of microscopic particles and beads for bioremediation (heavy metals,[98,99] hydrocarbons,[100] crude oil,[101] surfactants,[102] aromatic compounds,[103] etc.). Particulate microtraps that can sequester motile bacteria from liquid suspensions have been demonstrated.[104] In the former applications, besides the aspects of simplified handling, it was shown that immobilized microbial consortia always outperform free floating microbes in terms of operational temperature and pH range that can be sustained, as well as effectiveness of remediation.

The more immediate microbiological context is addressed by droplet micro-[105] or millifluidics,[106] in which either the generated droplet itself can provide a microniche, or droplet generation and further processing be used for the encapsulation of individual or multiple microbes and consortia.[107,108] Pico- to nanoliter chambers and encapsulated droplet communities hold promise as versatile tools to address both cultivation within the droplet[109] and spatial separation across droplet mixtures with varying individual community composition. This can be seen, for example, in the exploration of drug resistance of chronic infections, in the search for potential antibiotics, and in the study of cross-kingdom dynamics.[71] Taking this approach one step further, through synthesizing vesicles with multiple compartments, sequential or stepwise release of several components can be realized,[110] functional artificial cells can be obtained as a new type of biosynthetic reactors,[111,112] or vesicles can be arranged into higher-order structures using optical tweezers.[113]

Similarly to particulate materials, fibers also enable the cultivation of microbial consortia on their surface, encapsulated in hydrogels, or contained within a liquid carrier medium. For example, encapsulation of living *Escherichia coli* has been shown in electrospun fiber, assembled into microfibrous membranes.[114] *E. coli* encapsulation in hydrogel microfiber produced by microfluidic technologies was used for spatial separation during in vitro studies of the intestinal microbiome.[115] Beyond such studies, classic materials such as membrane hollow fiber used for ultrafiltration,[116] or glass hollow fiber can be employed to replace planar microfluidic elements, switching from *lab-on-chip* to *lab-in-fiber*.[117] Combining the optical functionality of microstructured glass fiber with micro- and nanofluidic technology enables optofluidic sensing and manipulation,[118] for example, for the label-free detection of viruses,[119] or for high-throughput proteomics such as required in the study of more complex microbial communities.[120] Also noteworthy, is that optofluidics can be performed in fiber as well as on chip. Besides acting as immediate containment or an arena for microbial species it appears that the most fundamental interest in optical fiber remains with its optical performance. In the present context, this means that optical fiber probes (lab-on-fiber, to be differentiated from lab-in-fiber[117]) and fiber light guides (e.g.,[121]) present important components for implementation with microbial arenas, in which they enable spatially and temporally controlled optical sensing, stimulation and manipulation. An intriguing opportunity lies in the combination of optically functional fiber with encapsulation fiber, hollow fibrous membranes, and textile processing for the generation of 3D microbial arenas with optical readout, tunable illumination gradients, tailored spatial segregation, and controlled release of auxiliary chemicals.

3D synthetic microbial arenas can be obtained through various processes. Often, porous materials are used, providing certain pore sizes and surface properties to house microbes, separate cells or capture biomolecules. As an example, cryogels are fabricated from appropriate monomers or oligomeric/polymeric precursors in semifrozen liquid media in which ice crystals act as porogen and thus as templates for the shape and size of the interconnected pores which appear after thawing.[122,123] Incorporation of specific binding ligands can be used, e.g., to isolate certain bacteria from media, as shown for mannose-functionalized cryogels for the catch and release of *E. coli*.[124] In analogy to the examples outlined above, microbial consortia immobilized in cryogels have been revealed to exhibit higher efficiency in degrading pollutants as compared to freely suspended cells.[125] Alternatively, all-inorganic matrix materials and, in particular, vitreous silica are available for the incorporation of living microbes through sol–gel chemistry.[126]

Putting the microbes themselves to work, engineered virus dynamics have been used for templating nanoscale architectures, for example, for nanoscale electric wiring of materials with poor electronic conductivity in battery components.[127] One step further, genetic circuits have been reported for the direction of amyloid production from *E. coli* into multiscale structures, featuring secondary nonliving components such as metallic nanoparticles or quantum dots for optical feedback.[128]

Progress in printing technologies and additive manufacturing processes enabled the fabrication of more complex structures from polymers,[129,130] glasses,[131] ceramics,[132] and biological tissue and organs[133,134] with centimeter dimensions down to micrometer resolution. 3D-printed scaffolds may readily be equipped with internal physical or chemical gradients.[135] Inks containing living bacteria (so-called functional living inks, "Flinks") enable literally living materials with application in biocatalysis,[136] or as self-healing or self-growing material.[51,137,138] At the same time, such printing strategies allow for selective encapsulation of bacterial communities in adjacent microcompartments in order to control their interaction.[139] The latter is enabled by two-photon polymerization (2PP,[140] also referred to as multiphoton lithography (MPL)[141]). 2PP has become a common tool for preparing 3D scaffolds for tissue engineering,[142] but also to microfabricate an almost limitless range of arbitrary geometries, thus offering new opportunities to rapidly

prototype complex architectures for microfluidic and cellular applications.[139] Structured hydrogels can be tailored for sensitivity to environmental changes, to undergo volume changes in response to pH-value, ionic strength and thermal triggers or to be light-responsive.[143] For example, 3D-printed hydrogels with embedded bacteria have been used to study chemical communication and quorum sensing in real time.[144,145]

5. Functionalization: From Physical to Chemical Stimuli

As noted in the previous sections, in order to enable control of complex consortia in cocultivation an overarching function of artificial microbial arenas is the ability to provide adaptive environments. Present cocultivation studies rarely exceed three species due to rapid community breakdown. Superimposition of exogenous parameter fluctuations on community dynamics can be used as an additional tool to actively counteract or direct community behavior. The provision of such passive or active stimuli requires dedicated integration of functional materials within the arena's layout. In particular, materials with switchable chemical or physical behavior are of the most immediate interest, however, passive components such as light guides, electrically conducting elements, or certain topographic features (for example, reducing wettability or promoting biofilm formation) provide versatile tools for designing specific functionality. A broad variety of stimulus-responsive materials are presently being researched on different levels of maturity.[74,146] In **Table 2**, we list a nonexhaustive selection of examples which could be of particular interest for implementation with microbial arenas. Common to all examples, investigations concerning toxicity and biocompatibility, material sensitivity within the physiologically relevant regime of triggers and responses, long-term stability and, e.g., the ability for autoclaving will need to be taken into account in future research.

Materials capable of undergoing changes in polarity, wettability, or surface charge hold particular promise as their interaction with microorganisms and their role within a microbial arena can be (reversibly) tuned. Here, one specific class of materials which is receiving continuous interest are stimuli-responsive polymers.[74,146] These materials can undergo a conformational transition (for example, coil-to-globule) upon applying suitable external triggers like changes in pH-value,[147] temperature,[148] or irradiation with light.[149] For example, this allows for the release of chemical stimuli from carrier particles[150] or vesicles,[151] and the opening and closing of nanoscale valves[152] triggered by light. Reversible hydrogels have been reported which undergo changes in gelation upon varying pH-values or temperature.[153] Here, significant response can be obtained, e.g., at pH 7.8 when varying the temperature within a range of 23–55 °C.

Special attention has been paid to materials which respond to multiple triggers, such as combinations of pH-value and temperature or pH-value and light,[174] involving readout mechanisms such as changes in color or fluorescence.[175,176] With temperature as the stimulus, materials featuring so-called lower (LCST) or upper critical solution temperatures (UCST) are well explored.[148] In the case of UCST, upon increasing the temperature above a critical value the material becomes water-soluble. At present, only a few examples are known in which this phenomenon is observed in aqueous media.[177] In addition, both for LCST and UCST, the temperature at which the desired response occurs is often significantly above the physiologically relevant range. Therefore, the challenge for materials scientists is to design chemistries in which rather subtle changes in pH-value or temperature translate into sharp and rapid changes in the degree of swelling or solubility of a polymeric material. Currently, this most promising route is through temperature-responsive materials, where the transition itself occurs at a defined and chemically adjustable point. On the other hand, response to changes in pH-value mostly still requires rather harsh conditions, notably acidic or basic solutions in contrast to the physiologically relevant range of approximately $5.5 < \text{pH} < 7.4$. Some examples can be found in drug delivery approaches where local tumor acidity promoted disintegration of suitable delivery vehicles.[178] The use of zwitterionic polymer coatings enables tailoring of bioadhesion:[179,180] by variation of polymer end groups on a coated surface, the adhesion of proteins (as the first stage of settlement) can be controlled in a wide range.[181] A way to circumvent the aforementioned problem is therefore the design of polyampholytes which exhibit a defined point of charge inversion where, attractive (electrostatic) interactions can be turned repulsive—initial examples demonstrate the reversible adsorption of polyelectrolytes or small molecules onto model hybrid nanoparticles.[182]

As maintaining either the response temperature or the targeted pH-value within the physiological range remains a challenge, other parameters become increasingly important, such as enzymatically mediated changes in polarity, ionic strength, biochemical gradients, or the presence of a certain metabolite.[183] Effects of topography, chemical and physical functionality were simulated in so-called liquid infused slippery surfaces (LISS).[184] Here, a surface is overlaid with a thin layer of liquid which is immiscible to the target (e.g., repelling) liquids. LISS can be produced from textured or smooth surfaces, polymer gels, or nanoporous glasses, and can adapt to external stimuli and environmental changes such as mechanical stress or strain, and magnetic and electric stimuli. As further examples, host–guest self-assembly of functionalized polycation/polyanion polymers can be used for visible-light-responsive surfaces with reversible switching between antibacterial and bioadhesive states.[185] Polyglycerol conjugated spiropyran immobilized on a surface responds to irradiation by light of various wavelengths in reversible switching of hydrophilicity accompanied with a change in nonspecific protein adsorption and cells adhesion.[186]

6. Summary and Outlook

The study of microbial consortia offers exciting challenges for materials research. In particular, the generation of complex artificial habitats—although a top-down subject—is largely limited by material-related questions: How does a given material selectively affect the dynamics of microbial consortia? How can advanced materials be structured, on multiple length scales and in different dimensions, so as to provide spatiotemporal control

Table 2. Examples of stimulus-responsive materials for generating adaptivity in microbial arenas.

Application	Material response	Response timescale	Reversibility	Example
Optical stimulus				
Antibacterial surfaces	Surface polarity	s	Reversible	Functionalized copolymer films[185]
Capture and release of microbes	Volume changes	min	Reversible	3D-printed photo-crosslinked hydrogel protein microstructures[143]
Multiresonant optical surface scattering	Light emission	ms	Reversible	Nanoplasmonic structures[154]
Spectral conversion	Light emission	ms	Reversible	Light-converting dyes[155]
Photothermal heating	Local temperature change	s	Reversible	Functional glasses[156]
Mechanical stimulus				
Generation of physical forces	Vesicle collapse	s	Irreversible	Reporter genes on *E. coli*, *Salmonella typhimurium*[157]
Generation of physical forces	Acoustic cavitation (vesicle collapse)	µs	Reversible (irreversible)	Nanoporous hard-shell polymer vesicles[158]
Structural reconstruction and growth	Mechanoradical polymerization	min–h	Irreversible	Crosslinked hydrogels[159]
Targeted illumination	IR-light emission	ms	Reversible	$CaZnOS:Nd^{3+}$[160]
Chemical stimulus				
Surfaces with switchable charge	Invertible surface charge	s–min	Reversible	Polyampholytes, block copolymers[161,182]
Control of protein adsorption	Switchable charge stage	min	Reversible	Surface-grafted carboxybetaine functionalized polymethacrylates[162]
Electrical stimulus				
Control of bacterial cell adhesion	Surface charge	min	Reversible	Electrochemically switchable SAM mercaptoundecanoic-acid tethered to gold surface[163]
Electroactive scaffolds	Swelling/de-swelling	min	Reversible	Graphene–hydrogel composites[164]
Immobilized electroluminescent dyes	Light emission	ms–s	Reversible	Functionalized zeolite L[165]
Thermal stimulus				
Triggered cell detachment	Interfacial hydration of hydrophilic segments	min	Reversible	Zwitterionic sulfobetaine methacrylate-based copolymers[166]
Detachment of affinity bound bioparticles	Elastic deformation	min–h	Reversible	Thermosensitive macroporous hydrogel with affinity ligands[167]
Magnetic stimulus				
Magnetic surfactants	Polarity	ms–min	Reversible	Ionic liquids, chelated surfactants, polyoxometalates[168]
Switchable membranes	Membrane permeability	min	Reversible	Fe_2O_3-functionalized hydrogels[169]
Multiscale switchable topography	Surface topography	s–min	Reversible	Ferrofluid-containing liquid-infused porous surfaces (FLIPS)[170]
Multiple stimuli				
Encapsulation and release: ph, *T*	Changes in viscosity, gel stability	s	Reversible	Hydrogels[153]
Sensor materials: ph, *T*	Changes in optical properties	s–min	Reversible	Functionalized copolymers[175]
Wetting behavior: *E, T*	Surface polarity	h	Reversible	Thermally poled glass surfaces[171]
Biosensors and antifouling: light, pH	Changes in surface polarity and charge	S	Reversible	TiO_2/dendritic polymer hybrid films[186]
Magnetoresponsive release: *H, T*	Localized heat	min	Irreversible	Hybrid liposomes functionalized with Fe_2O_3 nanoparticles[172]
Multiresponsive uptake/release: pH, *T, H*	Swelling/deswelling	min	Reversible	Nanocomposite conetworks functionalized with Fe_2O_3[173]

E: electrical field; *T*: thermal field; *H*: magnetic field.

over the pertinent interface reactions? How can responsiveness be implemented and which kinetic functions govern material response, buffering effects or switching ability? How do stimuli transmit into the consortium?

Materials with diverse functions and their associated manufacturing processes allow for the fabrication of microbial arenas as habitats for one, two or multispecies communities. Simulating complex environmental conditions, such arenas

will facilitate the elucidation of functional interdependencies, overarching interaction patterns and requisites for microbial balance. Stimuli-responsive and release-materials enable the interactive variation of exogenous parameters, for example, surface pH-value, local charge, wetting behavior and the availability of light. This sets the scene for the creation of adaptive arenas in which readout technologies, machine learning and stimulus generation are combined, for example, for stable cocultivation, or for community training and directed evolution. In return, new ideas will be generated for materials which control microbial dynamics, for example, through antimicrobial functions and nonadhesive or antifouling surfaces.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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