Probiotic design

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In response to advances in medical fields that now understand the integral role that bacteria play towards human health, this research proposes a novel probiotic design approach towards designing healthy buildings in relation to beneficial microbes. This research fundamentally challenges modern approaches to healthy buildings that assume fewer microbes as the default healthy condition.

Probiotic design builds on the contemporary understanding of the microbiome and the need for re-introducing environmental microbial diversity in to buildings. The research uses an interdisciplinary approach between microbiology and architecture which aims to develop living materials embedded with beneficial bacteria for buildings to directly shape the indoor microbiome towards a healthier microbial condition.

This approach utilises a mix of in vitro and in silico methodologies to explore the design, fabrication and survival of living probiotic materials which are then scaled up to the building scale as a series of probiotic tile surfaces and installed in a test space to monitor their effect on the indoor microbiome.

The research demonstrates evidence of a successful methodology for integrating viable bacteria into ceramic and concrete materials which are then proved to inhibit the growth of pathogens and in their ability to directly increase environmental microbial towards healthy indoor microbiomes.

Introduction

As we consider the design of future cities, the current viral pandemic has brought microbes and health back to the forefront of scientific and political minds. It has also put the microscopic, unseen world right back at the top of the public fear list. Microbes, as they were called in the past, are pathological ‘germs’. They are feared as bringers of illness and even death. Yet, before the pandemic, an emerging shift in the understanding of microbes and their relationship with human health has been unfolding, with important implications for the way we design our future cities. Unlike previous instances, whereby threats of infection and pandemics have been understood in relation to the presence or abundance of pathogenic microbes, the emergence of new twenty-first-century pathologies appear to be linked not to the presence but to the absence of microbes from our bodies and the environments we inhabit. It appears that in order to design healthy buildings, we need more microbes, not less.

Emerging medical knowledge is now understanding how ‘missing microbes’ from our bodies are playing a role in the recent emergence of allergic and inflammatory

diseases observed in developed urban environments.[1] Within a wider discourse, these so called 'pathologies of absence' have been attributed to an over-reliance on fundamentally antibiotic philosophies and approaches that have gone too far.[2] The same appears to be true in relation to cities. The relationship between architecture and microbes is historic, but has predominantly been based on negative associations of microbes. As a result, the discourse of designing healthy buildings and infrastructure has been dominated by attempts to remove microbial presence in buildings. Along with removing disease-causing microbes, however, such approaches also eradicate other microbes that are non-pathogenic and are essential for normal, resilient immune function. This emerging understanding comes alongside developing knowledge of the human microbiome and a contemporary medical understanding that not all microbes are pathogenic; many are benign, and some are beneficial, even essential, for health.[3] While contemporary approaches in the medical and environmental fields are embracing a probiotic turn,[4] architecture is yet to do so. We have argued previously that existing architectural approaches towards health are still rooted in a medical understanding associated with the antibiotic turn.[5] We propose a probiotic approach to the built environment as a way to align the field of architecture with the contemporary medical understanding of the human microbiome and health. This article presents this approach from a design perspective and suggests the relevance of biodesign as an existing area within architectural research. Through its fundamental approach of designing with living cells and systems, biodesign may be well placed to develop a probiotic approach to architecture.

The emergence of biodesign as a research agenda within architecture and design over the last two decades has been primarily driven towards contemporary agendas of sustainability and wider climatic discourses. At the material level, designers have looked to novel advances in biotechnology and biomedical fields for new approaches to creating sustainable materials and new ways for designing and building projects that utilise microorganisms and living cells.[6] Biofabrication, whereby materials or objects are 'grown' or made using living organisms, is seen as beneficial compared to current material production techniques. It serves primarily as an alternative to non-renewable, fossils-based material technologies that are energy intensive and/or produce harmful environmental agents. A selected body of work in this field looks to maintain the living element of the design as an ongoing condition. Attempts to use organisms such as bacteria and algae cells have looked to improve the performance of buildings in terms of structure and self-repair,[7] energy use, and the potential for energy

production and CO2 absorption through photosynthetic organisms integrated into building façades.[8]

Beyond the sustainable and climatic advantages of biofabrication, such approaches that involve co-creation with living organisms, have spurned new interdisciplinary and collaborative design processes. Designers are engaging and working within the disciplines of biology, chemistry, and materials science alongside iterative design methodologies, resulting in new and diverse forms of material expressions, aesthetics, novel product ideas, and new paradigms for architecture. In the conceptualisation of buildings, the designer's role has now shifted from being the 'specifier' of existing or fully developed materials to becoming the 'active maker' of new material proposals.[9]

In full support of the above agendas, this project expands to a new area for research within the fields of architecture, material design, and biofabrication, relating to emerging knowledge of the indoor microbiome and its relationship with human health in the built environment. Research on architectural design from the perspective of beneficial or missing microbes is limited. This project looks to take the first steps towards designing strategies for directly (re)introducing beneficial bacteria in buildings. It looks to raise awareness of, and work alongside, the contemporary medical understanding of the beneficial roles that microbes play in health, aiming to drive a similar change in the way we consider materials and microbes for use in the built environment. Understood as a small part of a much wider, urban-scale approach that is required to address this issue, the probiotic design approach developed here identifies materials and surfaces in buildings as niches for beneficial microbial cultivation. It unfolds as a direct method to encourage exposure to good microbes in buildings.

This article begins with a summary of the current understanding of the microbiome of the built environment (MoBE) and its relation to health. It specifically focuses on areas where design, especially when it addresses the material condition, can play an important role towards designing direct interventions that can make our built environment healthier through increasing beneficial microbial exposure.

The article then presents an initial and highly experimental body of work that explores this area as a starting point for developing probiotic design through probiotic materials that are manufactured to purposely grow beneficial bacterial strains on and within them. The research targets the three primary reservoirs of microbial presence in buildings: in the air, in the water systems, and on surfaces. It uses these conditions as a starting point to understand how design can modulate and drive the indoor microbiome towards a healthier state. This is explored as an interdisciplinary body of

work that brings together expertise in architecture, the built environment microbiome, and antimicrobial resistance. The article presents a multi-scalar approach using an interdisciplinary, collaborative mix of scientific methodologies and lab work (including microbiology and material science), alongside architectural and artistic design.

The work initially focuses on surfaces as a vector between the microbes on and in the human, and those in the building. This approach builds on the condition of fomites defined (currently in negative terms) as an inanimate object or surface which, when contaminated with or exposed to infectious agents, can transfer disease to a new host. By flipping this condition, the work designs surfaces to act as a source of good microbes that can ‘re-contaminate’ the body with the environmental microbial diversity that is currently missing in cities. This research looks further into the future and asks: Can we move from considering materials that are not detrimental to health to actually designing materials that are beneficial for health?

The main aim is to design hybrid living materials towards active buildings elements that act as a direct source of beneficial environmental microbes to the indoor and potentially the human microbiome. In addition, such approaches may facilitate beneficial biological functions, ranging from inhibiting pathogens to reducing volatile organic compounds (VOC) and metabolising other harmful chemicals into harmless ones. The work is presented in three stages. At the microscale, a mix of in vitro and in silico methodologies are used to explore the design, fabrication, and survival of living probiotic materials. These are then scaled up to create probiotic surfaces using living tiles in stage two. Finally, in stage three, the probiotic living tiles are installed in a real-world test space to monitor their ability to directly manipulate the indoor microbiome towards a beneficial condition.

The overarching aim of this article is to better engage the field of architecture with the field of the built environment microbiome, specifically in relation to beneficial microbes. As such, the text engages with a design context, focusing on the design elements of the work. When possible, it tries to avoid overly scientific or microbiological terminologies. But it also suggests that a common dialogue between the different fields is necessary. For similar reasons, the text does notinclude raw or numerical data from the microbial testing which would be beyond the scope of the article. Instead, the microbiological results are summarised through text and images from the three stages of the work to describe the design approaches. Taken together, these approaches constitute a first step towards designing beneficial microbiomes. Specific microbiological data and results from the three stages will be published as separate papers to engage with the scientific community, hoping to highlight the role that design can play in this field within that community.

Design for the indoor microbiome

The MoBE describes the complex ecosystem of microbial communities that exist as part of everyday life, both on the surfaces and in the air of our buildings. Within buildings, microbes are found in three main reservoirs of air, water, and surfaces. Microbes here include bacteria, fungi, viruses, and protozoa. The types and constitution of these microbes are made up from those living in the building, those that are bought in from the outside, and those that come from occupants. In the same way that humans have a microbiome that is unique to the individual, literature shows that the constitution of a building's indoor microbiome is equally unique. This is influenced by a number of external factors, including building location, climate, surrounding geography, and building occupants (including humans and pets).[10] As such, the building's indoor microbiome is time based; it will change over time.[11]

Investigations of the MoBE and more specifically the indoor microbiome (IM) are emerging research areas, centred around the principle that the microorganisms that exist in buildings can directly impact on human health through their effect on the human microbiome.[12] Alongside data that supports this for many types of infectious microbes, more recent reports suggest beneficial effects on health from microbial exposure in the built environment. This is primarily in relation to normal and healthy bodily functions, including metabolism, immune function, and cognition. Evidence has also shown that building design influences the diversity and structure of the IM,[13] suggesting a direct link between building design and health. Understanding the mechanisms between design applications and their effect on the types of microbes, and those that we are exposed to, in buildings will be key towards developing design approaches for heathy buildings that can encourage exposure to potentially beneficial microbes.

There currently exists no role for microorganisms in buildings. Within western cultures, a general fear of microbes, particularly of bacteria, means that any known or suspected presence of microbes in buildings is negative; it is typically associated with a fear of infectious disease or illness. While this research looks to engage with beneficial microbes, it does so critically by acknowledging well-known negative cases of microbes in buildings. Microbial presence in this sense is often a signifier of a building problem or failure, usually a leak or damp. A range of allergic and non-allergic related illnesses, including asthma, rhinitis, eczema, and respiratory problems, have been associated with 'sick buildings' that exhibit damp or have water-related damage. Damp building conditions are known to enable the growth of bacteria, mould, and other microbes, whose visual presence is related to a building defect.

Alongside this, the link between exposures to microorganisms as pathogens in buildings and human health is well established and understood for many types of infectious bacteria, viruses, and fungi. Transmission routes primarily relate to inhalation from airborne microbes re-suspended from room air, or those aerosolised from building water systems. These relate to human exposure via routes to the respiratory system or translated to the gastrointestinal tract. An additional major transmission route is via fomites whereby microorganisms present on surfaces are transferred to the human body via direct contact which is then spread by touching the mouth, eyes, or nose. The material condition is of particular importance. As was also evident with the recent coronavirus, microbial communities can survive for extended periods of time on indoor surfaces.[14] This has been especially significant in relation to understanding hospital acquired infections and the more recent global issue of increasing anti-microbial resistance (AMR).

Although we rely on biocidal chemicals and stringent cleaning routines, in reality sterilising a surface is merely a temporary measure, since recolonisation takes place within minutes. Evidence also shows that the sterile surface becomes an uncontested one for opportunistic microbes to grow, and antibacterial chemicals themselves may also promote resistance to clinical antibiotics.[15]

Urban dysbiosis describes how urban lifestyles are degrading the microbes in our bodies. Microbiomes sampled in urban areas are lower in mass and significantly less diverse than microbiomes sampled in rural areas.[16] This diversity that exists in nature, and that of which the human immune system evolved with over millennia, is slowly being lost from our urban environments.[17] As more and more people move to cities, and as we spend up to 95% longer time indoors,[18] we are less and less exposed to the microbial diversity associated with nature. Conversely, we are increasingly exposed to the human-dominated microbes that exist in buildings.

Most importantly for advances in architectural discourse is that building design plays a key role in both the types and amounts of microbes that constitute the indoor microbiome of a building.[19] By understanding these relationships, design can play a role in shaping healthy microbiomes. This understanding of microbes raises new questions about how we might design buildings and spaces in relation to microbes and how we interact with them. A probiotic approach would require a more balanced, even pro-microbial philosophy towards the built environment. It would consider completely new strategies that could reduce exposure to pathogens, but also increase exposure to beneficial microbes.

Since this area of research is currently dominated by the related scientific disciplines, the input of designers is both necessary and called upon.[20] Conversely,

rendering research in this field more relatable and understandable to designers is equally important.[21] The biodesign or 'growing design' approach used here may be well suited as a design methodology to contribute to this field.[22] The challenges are many, ranging from technical issues of working with living organisms to overcoming the engrained negative perception of microbes. Designers in this field tend to be mediators of multiple disciplines. They often work in multidisciplinary manners with good levels of understanding of biology and biotechnology. As such, they are well placed to understand and build on the scientific approaches, methodologies, and leading data in this field. Furthermore, previous projects utilising living organisms have tended to develop designer attitudes that attribute a higher value to the material and the living organism associated with it.[23] Materials that contain living organisms tend to have their own agency. Hence, biofabrication utilises a co-design approach with the living organism, where the needs of the organism drive a bottom-up process. Biofabrication-based approaches also place value on the experiential and aesthetic qualities of materials that extend beyond a narrow focus on functionality or performance. Such an approach can instigate cultural and social acceptance for probiotic materials that might challenge pre-existing imaginaries of microbes.

Probiotic design

The research aims to develop a novel probiotic design process towards creating healthy indoor microbiomes. This begins with the development of Probiotic Materials for use in buildings that are designed to contain, catch, and shed beneficial microbes. These are proposed as hybrid living materials containing viable benign strains of bacteria that are beneficial for health. This work looks beyond the typically inert and sterile nature of contemporary architectural materials towards living materials that have an inherent biological agency. Materials found in the natural world, including wood, bone, and skin, comprise the structural matrix's containing living cells. Through this living agency, these hybrid materials are able to perform dynamic biological functions, including energy harvesting, sensing, and actuation. They also demonstrate resilience to stresses through self-repair, adaptation, and growth. As such, natural living materials can survive for years, even millennia, in fluctuating and stressful conditions, under which many inert architectural materials cannot.

The notion of creating new types of living materials has a precedent in the field of biodesign, including approaches who have sought to explore the potential for the design and application of alive or semi-living materials at a range of scales, from apparel to products and buildings. This project builds on previous work in the area of biofabrication involving design with living organisms. It shares similar features with both

the ‘growing materials’ and the ‘DIY materials approach’.[24] Under this categorisation, this work perhaps most closely utilises the ‘DIY new identities for conventional materials’ approach. As such, the work presented focuses on new production techniques of existing materials, aiming to define new identities for existing materials. From an architectural perspective, this approach has a recent precedent in the bioreceptive design approaches developed by the author, whereby designing building materials in terms of their material bioreceptivity and surface geometry supports the growth of photosynthetic organisms on the outward-facing façades of green buildings.[25]

This research turns to the interior of the built environment and the indoor microbiome. Hence, the design switches its focus from outdoor environmental considerations of rainwater collection, sunlight, and shading, to indoor environmental factors, including airflow, surfaces, moisture, and human behaviours. The work initially addresses non-structural elements and surfaces to create an invisible microbial fabric that envelopes the structural building (Fig. 1). This functions as a substrate for, and source of, diverse microbial communities as an active addition to the built environment microbiome.

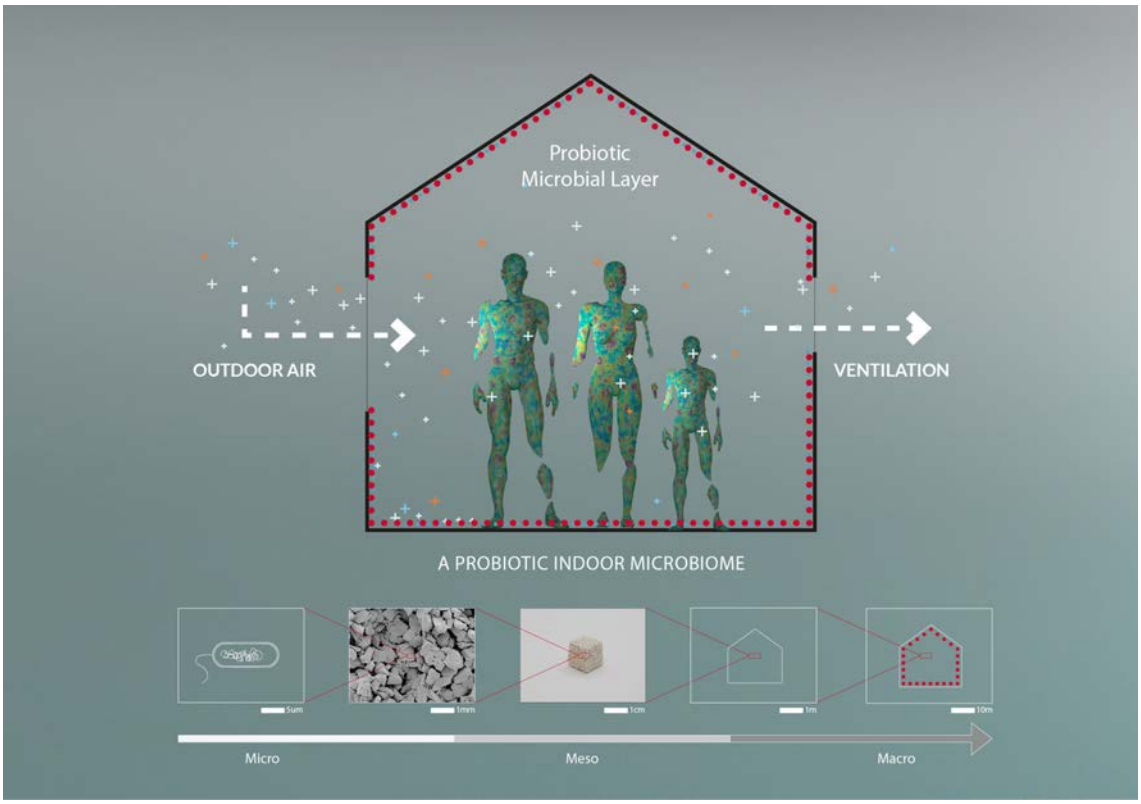


Figure 1: A probiotic Indoor microbiome approach using a multi scale design process

The work follows a multi-scalar approach, ranging from the micro-scale of the organism and the meso-scale of building components to the macro-scale of the environmental

and microbial interface between humans and the building. The first phase addresses the cellular micro-scale of the individual Bacillus cells and their larger colony formations. Bacillus subtilis are rod-shaped bacteria which divide in one plane to produce streptobacillus chains. These can be manipulated to form biofilm which can in turn be tuned to switch between dormant endospores and active germinating cells. From the millimetre upwards, the physical properties of the material are considered from an architectural perspective in the second phase, which examines the strength and feasibility of fabricating larger meso-scale elements. From the centimetre upwards, the third phase addresses the macro-scale that involves interactions between the human scale and the conditions of the built environment. A series of architectural components are designed to act as a source and sink of beneficial microbes that can prevent human exposure to pathogens in buildings.

Despite its scientific context, this work is fundamentally led by design and continually considered through an architectural lens. The occasionally complex microbiological methodologies were always developed according to the needs of the designer. These included architectural requirements for feasible use at building scales, regarding properties such as strength, cost, and method of fabrication, but also considerations of aesthetics, maintenance, and user perceptions. While this complex entanglement of factors seems common to architects, it remains out of scope when one follows a purely microbiological or material-science-led approach. From an architect's perspective, the microbial methodologies used here as part of the design process might be comparable to the way that an architect might engage with a structural engineer or surveyor. Future, probiotic design of healthy buildings would certainly involve the need for microbiologists as part of the design process.

Micro-scale: microbial-led material design

The initial phase of work involves a material and microbial exploration phase towards the identification and development of materials which have the potential to act as substrate scaffolds to support living cells embedded in the material. This is explored initially at the micro-scale of the material-bacteria interface. Perhaps best framed in opposition to antimicrobial materials which aim to prevent microbial colonisation, here the work looks instead to design materials that allow for, and even augment, beneficial microbial presence. In this manner, materials are considered and designed according to the requirements of the microbes defining the microbial-led process. In the studio, physical and chemical properties of the materials are targeted, characterised, and tweaked according to literature values for the chosen strains or communities of bacteria. Then in the laboratory, microbes are embedded into the material volume and

quantifiable microbiological testing is used to provide feedback for success, failure, and iteration.

Feedback and success at this point was driven by the biological notion of microbial survival. This primarily refers to whether the bacteria are able to survive once embedded into the material, or what we call material-microbe viability here. One major challenge for living materials and their application to architecture is the requirement for continuous nutrients and water, both of which are limited in buildings. Without this ongoing maintenance, the living agency of the material is likely to perish within a short time. It is a key aim of this research to avoid expensive or intensive systems to supply water and nutrients. A comparison might be drawn here in respect to green walls which, despite their benefits, have not been widely adopted. This is predominantly owing to the high costs of artificial irrigation systems and ongoing maintenance that is required to keep them alive.[26] Instead, this approach looks to build on strategies for survival that microbes have evolved for, even in the unlikeliest and most extreme environments. We work here with bacterial strains and communities that have the ability to survive in dry indoor conditions by transitioning to spores, a dormant and extremely tough low-energy state. Biological research in extreme environments has shown how bacterial spores can lie dormant indefinitely, and withstand extremes of temperature, pressure, pH, and UV light. We worked specifically with a strain of Bacillus subtilis, a benign bacterial strain commonly found in soils and the gastrointestinal tract of animals. This species was selected due to its probiotic potential, its ability to produce antimicrobials with multiple modes of activity, and its capacity to generate molecules that prevent the adhesion and accumulation of other microorganisms.[27] In addition, B.subtilis are able to form spores that can remain dormant for hundreds of years, staying highly resistant to alkaline pH, heat, and other environmental stresses such as dehydration.[28]

Finally, design plays a key role in a sort of microbial computation here, as it aims to stimulate the bacteria to undergo this transition, and remain ready to be reanimated in response to a trigger such as the presence of water due to flood or damp, or the presence of a pathogen. This first micro-scale design stage looks to achieve phenotypic transition from motile single cells to a surface-based biofilm, and finally sporulation. This bioaugmentation of the substrate/organism interface drives the development of a resilient probiotic material. The work presented in this section explores a microbial-led material strategy towards the self-assembly of B.subtilis biofilms within the material matrix to create a bioanimate material that has the resilience to remain viable in normal indoor environments without the need for nutrients or water.

Material selection

In considering potential materials for probiotic design, we decided to build on the ‘new identities for conventional materials’ approach for two reasons. First, we aimed to explore the use of materials that are already common in architecture but can be made porous during the manufacturing process. Hence, we looked at ‘mixed materials’ such as concretes and ceramics, whereby variations in aggregate size, water/binder ratios, and compaction can result in different, and even gradient, pore sizes. Plastics and metals were also explored using 3D printing, where the resultant material porosity can be defined through the 3D computational design of porous lattices and tweaked through the manufacturing process. Second, a core aim of the work was to avoid expensive materials that rely on costly, pharmaceutical-grade materials, such as those that can only be made in small quantities, whose scaling up would be limited by either available technology or cost. Hence, we aimed to develop materials that are more feasible for real-world use in the built environment.

Figure 2: A range of materials tested for material-microbe viability

Based on these criteria, a range of materials were selected for initial exploration: ceramics (well known in buildings, typically used in wall and floor tiles, and other objects); concretes (well known in buildings, typically structural, but also used non-structurally on floors, walls, and panels); 3D-printed plastic (Nylon PA2200 via SLS technology, potentially to be used for components and objects); and 3D-printed

aluminium (via DMLS technology, potentially to be used for components, frames, and door handles). The initial exploration phase involved fabricating a range of samples from this selection akin to the tinkering stage in a DIY material approach. Material porosity was defined as a key property for successful bacterial integration and colonisation of material volumes. The specific pore size and distribution of pores throughout the volume generate enclosures or niches within the material volume, in which bacteria can physically attach. Optimisation of these pores allows for more favourable conditions for survival and growth.

1 cm^3 cubic samples were first mixed and fabricated in the design studio, and taken to the laboratory for inoculation with bacteria. These samples were then assessed for their probiotic suitability using the notion of microbial survival as the driver for decision making (Fig. 2). Through this process, we aimed to: define a methodology of dosage and fabrication to produce specimens of concrete, ceramic, nylon, and aluminium with target values of pH and porosity; characterise the different mixtures in terms of pH and porosity; assess the material/microbe viability following inoculation; and select best materials to undergo further biological survival testing and drive the design process.

Stage one: material/microbe viability

Material samples were fabricated in 1 cm^3 cubes chosen as suitable for microbiological assessment at this stage. Each material required its own bespoke method of fabrication to achieve porosity values close to those identified in the literature.

Porous Concrete samples were fabricated using a dry-mix mortar methodology. Aggregates of known particle size ranges were mixed with low water/cement ratios in an overall mix. This provided enough binder to coat the aggregates and bind them, but did not fill the interstitial pores, resulting in a strong porous material once cured. These mortar mixes were compacted into moulds. Three aggregate sizes were explored to achieve different average pore sizes for experimentation.

Porous ceramic samples were produced using aluminosilicate particles (Si02 & Al203) of known sizes, compacted into moulds, and bonded by glass during firing.

3D printed metal and plastic samples were designed computationally as volumes filled with triangular lattices with designated thicknesses resulting in 0.4 mm pore sizes between lattices.

Fabricated material samples were demoulded, weighted and then stored at normal room conditions for fourteen days. Samples were then assessed using scanning electron microscopy (SEM) to observe the average pore size of the samples.

Material samples were then taken to the laboratory for inoculation with B. subtilis cells. Samples were sterilised using an autoclave and stored under sterile conditions for inoculation. The B.subtilis strain was then grown in a liquid culture to a known ODI whereby 300 ul were hand pipetted into the material samples. Inoculated samples were then stored in a sterile manner for two days. Afterwards, samples were re-plated onto nutrient agar plates to assess material/microbial viability, as evidenced by observable growth (Fig. 3).

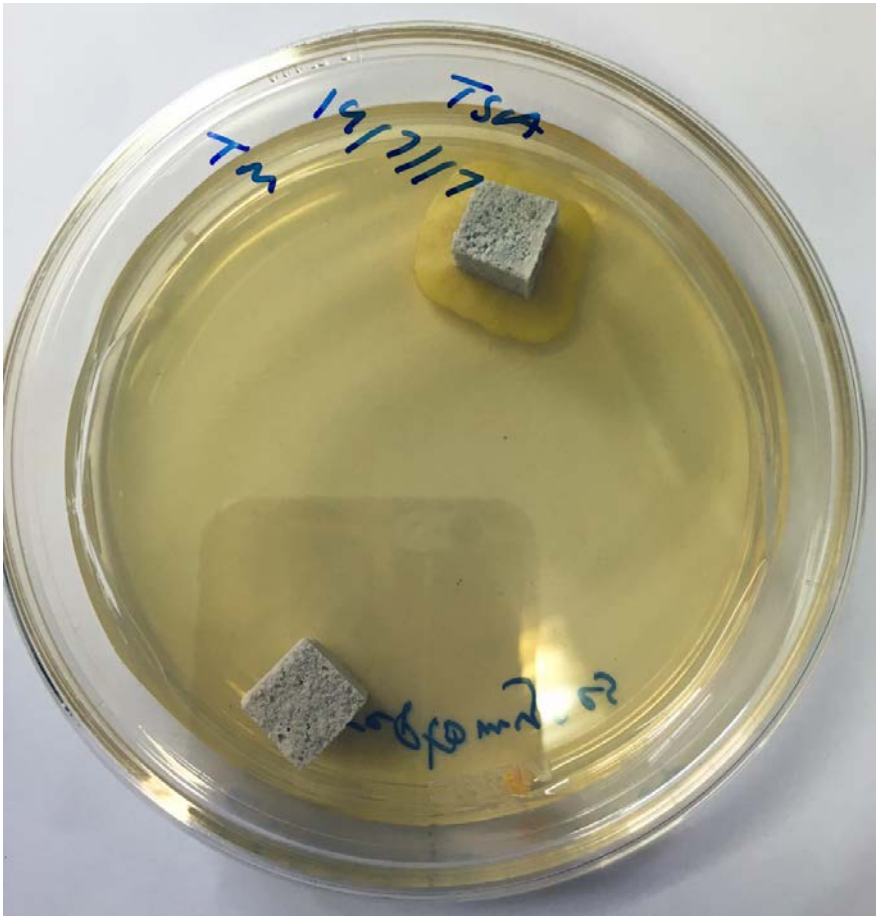


Figure 3: Testing material - microbial viability through regermination

Due to the costs and time required for further evaluation, only selected materials were taken forward for further testing. Materials that did not facilitate microbial viability were the first to be rejected. 3D-printed materials were also retired despite their potential, predominantly due the high cost of producing the large numbers of samples required for further testing. Since concrete and ceramic samples appeared to perform comparably at this point (Fig. 4), they were both selected for further testing.

Material	Av Pore Diameter	pH	Fabrication	Cost	Potential Use	Bacteria viable?	Summary
Concrete P02.5	33um	12	mix/cast	£	walls/floors/elements	no	Chemical issues
Concrete M02.5	36um	10	mix/cast	££	walls/floors/elements	yes	good
Ceramic M8	28um	8	mix/cast/fire	££	walls/floors/tiles	yes	best
Ceramic M10	55um	8	mix/cast/fire	££	walls/floors/tiles	yes	good
3D printed Nylon	0.4mm *	8	design/3D print	£££	components/handles	yes	too expensive
3D printed Aluminium	0.1mm*		design/3D print	££££	components/handles	not tested	too expensive

Stage two: microbial survival

These probiotic materials were then assessed for their ability to survive long-term desiccation stress associated with typical indoor environments. Material samples were fabricated, sterilised, and inoculated with B. subtilis cells, following the same methodology. Material samples were again stored under sterile conditions at room temperature as part of a controlled experimental procedure (Fig. 5).

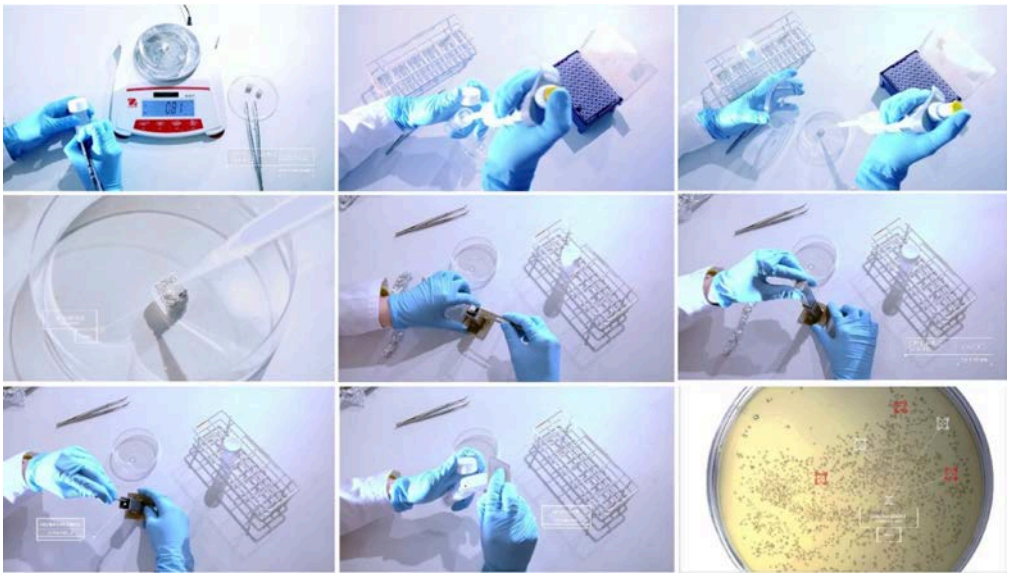
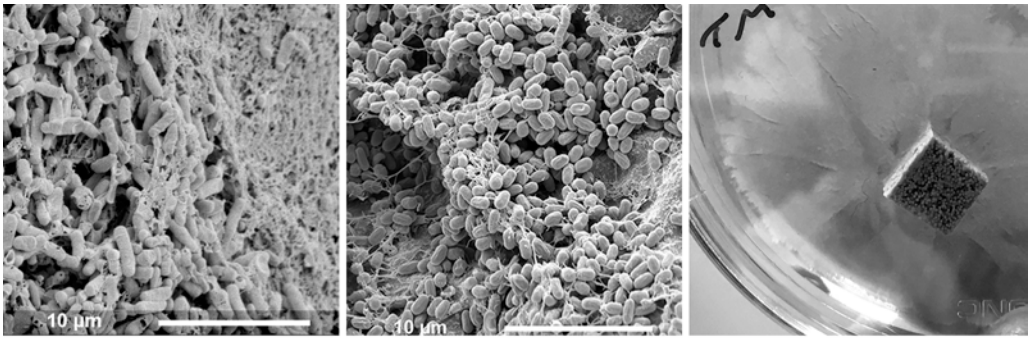


Figure 4: Microbiological methodology to determine microbial survival via cell counting

This time, a more rigorous approach to microbial survival was developed with a quantifiable methodology based on counting cells. During inoculation, a known number of bacteria were put into the material samples and a cell-counting method was developed to compare the number of cells extracted from the material samples. Using biological triplicate studies under sterile conditions, samples were tested at days one, seven, fourteen, and twenty-eight. Cube samples were put into a bespoke crushing

instrument made for the test to break apart the materials and access the microbes. Crushed samples were then washed out and vortexed to separate the biological cells from the material particles. These cells were then extracted and grown on nutrient plates. Afterwards, cell counting was used to determine the number of viable cells. SEM analysis was used again at the same timepoints to observe the Bacillus cells in the material over time.

The number of cells counted at each timepoint showed no significant difference. This suggests that Bacillus subtilis cells inoculated into the ceramic material were able to survive for one month (the maximum tested) without any nutrient or water restock. SEM analysis appeared to show the desired phenotypic change in state from germinated cells to spores over the same time period. Samples on days one and seven showed the Bacillus still in their germinated state; by day twenty-one, they appeared as spores (Fig. 6). Based on the known longevity of spores, it can be reasonably expected that survival and subsequent regermination would be possible after indefinite storage under normal indoor conditions.



Day 01 (Germinated cells) Day 21 (Sporulated form) Day 28 (replated)

This work demonstrates that building materials embedded with beneficial, healthy living functions can survive for extended times without maintenance or the need for water or nutrient restock. Longer-term testing and better understanding of the spore formation would be beneficial. In the future, this research could also assess the potential of directly embedding the bacteria in their spore form to the material matrix. This could simplify the preparation and growth phase of the probiotic material biofabrication process.

Stage three: probiotic action

The final stage of work at the micro-scale set out to assess the probiotic action of the hybrid material. Probiotic action was here defined specifically through the ability of the living material to inhibit the growth of a known pathogen. The beneficial relation to

health here follows the hypothesis that probiotic materials could reduce the pathogen load in a building, thereby reducing the likelihood of surface-acquired infection. In this case, we chose to test against Methicillin-resistant S. aureus (MRSA), a strain of S. aureus that has acquired broad antibiotic resistance and is an important health concern in hospital, domestic, and communal environments.

An initial test was undertaken by placing a disc of the living material on a culture plate which offered evidence in support of the hypothesis. Following this, triplicate inhibition assays were used to determine the ability of the probiotic material to inhibit MRSA. Three combinations were tested, including co-culture: B. subtilis + S. aureus; mono-cultures, B. subtilis + TSB; and NB + S. aureus. The data showed that S. aureus cells were below the limit of detection on the co-culture plate (with controls), suggesting that the probiotic material was able to inhibit the growth of MRSA.

In conclusion, in the micro-scale phase we developed hybrid materials that combine the strength of ceramic with the beneficial living properties of soil-derived bacteria, including antimicrobial-resistant mechanisms. We showed that these beneficial bacteria could be integrated into architectural materials (probiotic materials), survive over time, and prevent AMR bacteria colonisation.

Meso-scale: microbial-led surface development

This next phase of work involves scaling up the probiotic material developed in the micro-scale package to form architectural surfaces. At the scale of the mm upwards, I design a series of three-dimensional probiotic tiles envisaged as a tessellated biodigital living, wall-tile interface. These hybrid living surfaces aim to serve as an augmented invisible microbial fabric that envelopes the indoor environment and undergoes microbial exchange with the inner organs, nasal passages, and dermal layers of the human body. In this phase, the design focus shifts from the sub-millimetre material properties to those of visible scale geometries, from the mm upwards. At this scale, three-dimensional surface geometry plays an important role in determining how the hybrid living material interacts with the indoor environment and the human body. Probiotic surfaces now begin to interact with the building and its users. Extrusions and depressions, curves and folds are tested using Computational Fluid Dynamics (CFD) simulations in relation to air flowing past and over them. These surface variations, and subsequently the tiles themselves, become locally and spatially programmable. They are designed to augment microbial deposition and resuspension, to both collect and shed microbes in response to vectors of airflow, moisture, and human touch.

Knowledge and understanding of the indoor microbiome is incomplete, since it is still evolving. The majority of existing work on interventions that aim to change the

types of microbes in buildings has been driven by negative associations of microbes. Hence, it has looked towards removing or limiting microbial presence as the preferred outcome. From a microbial perspective, indirect approaches related to ventilation strategies, HVAC systems, and envelope tightness look to control or filter microbial presence in the air inside buildings. Direct methods include germicidal or antimicrobial chemicals and cleaning products or antimicrobial technologies such as UV irradiation.

Efforts to increase microbes in buildings towards a beneficial condition are comparatively less explored. While indirect methods are suggested as potential strategies within the literature, little has been tested. Direct methods to add microbes and design the indoor microbiome to a beneficial condition do not exist. But emerging work is now beginning to engage with positive microbial interactions, aiming to indirectly or directly increase beneficial microbes in buildings. In a similar fashion, indirect approaches look to strategies that can increase environmental microbes coming inside from outdoor sources. As the only real source of environmental microbes in cities is from parks and soils, much of this work looks towards increasing green space adjacent to buildings.

The surface design of the probiotic tiles was led through a series of three-dimensional geometry studies defined as typologies, which were conceived from multiple perspectives. A series of 150 × 1500 mm tile typologies with geometrical differentiations were modelled computationally to create microclimate variation on the surface in relation to airflow. In opposition to smooth surfaces, highly textural peaks and valleys create areas of micro-turbulence and eddies, as air flows past the surface. The surface roughness of the material and the geometrical features act both to trap microbes from the environment, but also to shed or re-suspend microbes pre-embedded in the material. These geometries were also driven by aesthetic considerations. First, tiling systems were required to allow for multiple tiles to be arranged in different manners and orientations to allow for interesting aesthetic variability (Fig. 7). This would also allow for spatial programming in relation to an environment that could be designed to shed more or less microbes, depending on the conditions required. Finally, highly detailed textures and geometries can create extremely tactile effects that encourage people to touch the tile surfaces. In this manner, the geometrical design was considered as a method for integrating the probiotic tiles in relation to the human body and behaviours, by encouraging human physical contact the tiles as a method of microbial transfer.

Figure 6. Three geometrical typologies from left to right; Typology A - Pitted Geometries, Typology b - Branching Geometries and Typology C - Creviced geometries .

Each of the geometrical typologies were then assessed by CFD software to model the flow of particles over the geometrical surfaces. This was used as a process to simulate the movement and flow of air, and subsequently microbes, over the surface of the geometrical typologies. It also offers an indication of how both the microbes in the material and other airborne microbes would relate and interact in relation to airflow in an indoor environment. In this case, microbes in the air are either free or attached to dust, or moisture particles that move around via air flow (ventilation or HVAC systems), or those re-suspended by occupant movement, or finally those that are transferred by touch.

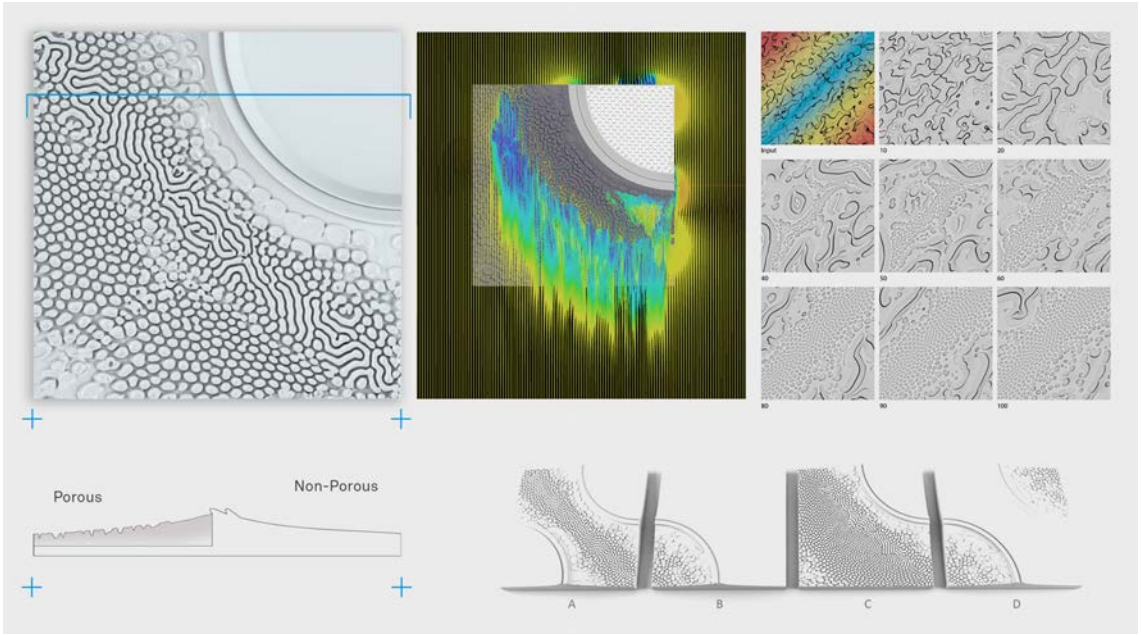


Figure 7 Geometrical typology A was developed using cfd simulations in to a tiling family of 4 types.

Based on the CFD analysis, typology A was selected for further development towards a tile set. The geometry was developed into a tiling system with four variations that tile together in multiple orientations to allow for geometrical variability, but also as a method to define how microbes might be spatially distributed. Textured, geometrical areas defined zones on the tiles where microbes would be inoculated. These areas would be fabricated using the probiotic material developed in the first phase.

Smooth areas were also introduced to the tiles. These areas served multiple functions. First, in relation to the CFD studies, these smooth areas allowed airflow to pass more freely and quickly, compared to the textured areas. The differentiation in friction served to create zones of turbulence and zones of quicker airflow which served to both shed microbes from the inoculated zones but also direct and trap microbes from the air, when applied to a multiple tile system. These smooth areas would be fabricated with a non-porous materiality which also gave more strength to the porous tile element (Fig. 8).

Based on CFD analysis, selected geometries were then chosen for fabrication testing using a novel casting system. The tile geometries were 3D printed using DFM technologies as positives, from which negative rubber moulds were produced, to allow for multiple tile casts. A two-layer rubber mould was produced using a delicate and extremely soft face skin made with shore 10 silicone backed with a more rigid shore 50 rubber. The face skin method allowed for successful demoulding of the delicate geometries, while the rigid back facilitated the compaction method required for the

ceramic and concrete material fabrication method developed in the first stage (Figs 9–11).

Figure 9.surface detail of probiotic tiles showing the textural, porous surface of the probiotic zone

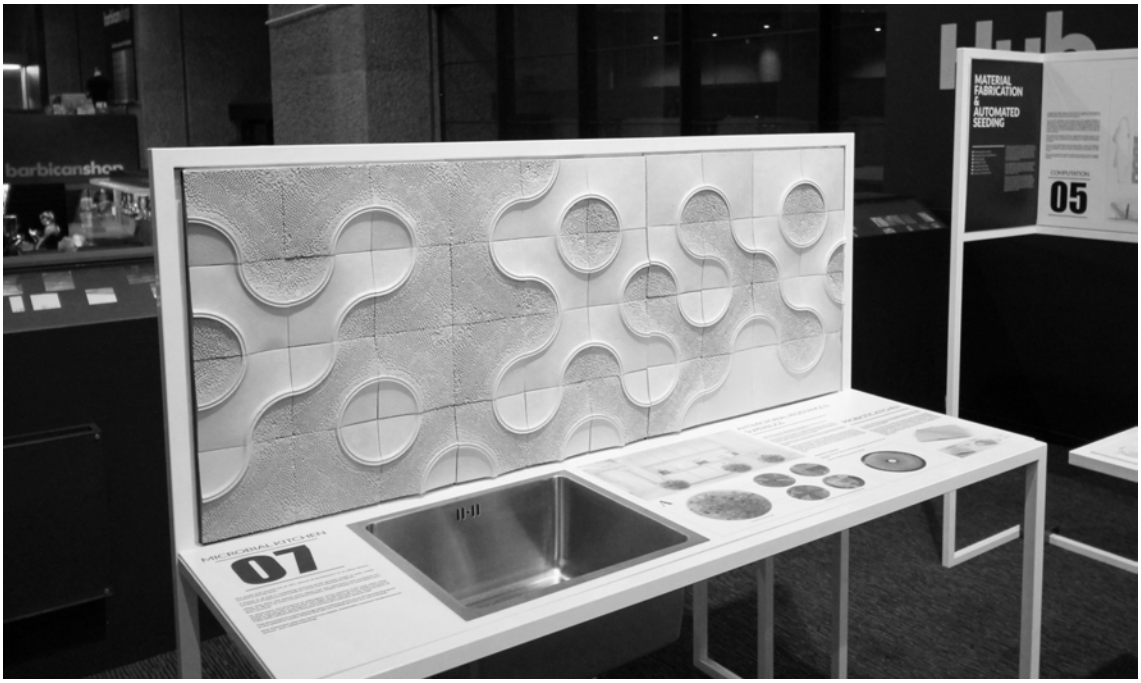


Figure 10. A 10x4 arrangement of ceramic tiles designed with spatially programmed probiotic zones. These tiles were Installed as an exhibition of the work at The Barbican In London as part of the Life Rewired festival 2019.

Macro-scale: probiotic environments

The final phase of work explores the potential for probiotic design to directly influence the indoor microbiome towards a more beneficial state. As such, it acts as a proof of concept for this approach and as a seed for further work in this area. Here, the focus shifts upwards to the macro-scale of the building, directly engaging the living material condition with the indoor environment and microbiome. This phase was based on the hypothesis that the introduction of the probiotic installation would have a direct influence on the indoor microbiome.

The research aimed to determine the effect of the probiotic installation on the indoor microbiome. It involved direct testing of the work developed in the previous phases by applying it to a real-world test space and using indoor microbiome measurements to monitor its effect on the microbiome of the space. To do this, a nine-week, time-based study was instigated. Using a before, during, and after approach, it aimed to directly assess the effect of installing the probiotic tiles in the space. This in turn built upon literature suggestions for using both culture methods and high-throughput DNA sequencing approaches, alongside environmental monitoring of temperature, humidity, and airflow. It also involved a drawn architectural survey to understand the microbial data in relation to spatial layout, fenestration strategies, HVAC systems, and occupant usage.

The research thus introduces the idea of a ‘microbial survey’ as an integrated part of the architectural design process rather than purely as tool for characterisation, as it commonly appears in the literature. It also looks into how these surveys can be used as a design driver by identifying both potential problems or challenges and areas that might be improved (microbially). In this sense, the microbial survey offers a snapshot of the types of microbes that exist in the space. But it also allows for insight into factors such as the spatial distribution of specific taxa, communities, and their relationship with building properties or occupant usage.

To date, research on the indoor microbiome has focused more on characterisation and less on its direct manipulation by design. While existing gaps in knowledge do not define what exactly constitutes a ‘good’ indoor microbiome in relation to the human microbiome, this stage of the work builds on literature that describes the beneficial effect of diverse soil microbes for human health. While testing this directly in relation to human health is beyond the scope of this work, we can use the distinction between human-associated microbes and environmental microbes as a way to test design. Since it is these diverse environmental microbes that are disappearing from indoor spaces, this approach serves partly as a potential method to combat microbial

loss associated with urbanisation. It might therefore be considered as a form of urban microbial rewilding.

In this phase, the research aimed to explore whether the introduction of a probiotic installation has an influence on the indoor microbiome. Due to safe working practices in relation to microbes, it was not possible to inoculate this installation with the Bacillus subtilis strain used in the micro-scale phase. In this phase, the tiles would be inoculated with a community of soil microbes obtained from a source of biodiverse vegetation in a rural Hertfordshire garden.

Within a limited budget, a series of 10 cm^2 sampling sites were selected in an attempt to generate data from a range of conditions within the space. Again, based on literature suggestions, a mix of horizontal and vertical surfaces were selected. Sites in close vicinity to the window and sites furthest away, as well as sites that were differentiated by the notion of high- or low-touch surfaces in relation to occupant use were selected to provide a range of results across walls, floors, surfaces, and materials (Fig. 12). No air sampling was undertaken during this work.

The research therefore aimed to: design, fabricate, and install probiotic prototype for the space; collect microbiological samples for a set period of time before, during, and after the probiotic material was introduced and subsequently removed; and identify any differences in the indoor microbiome by carrying out multivariate statistical methods.

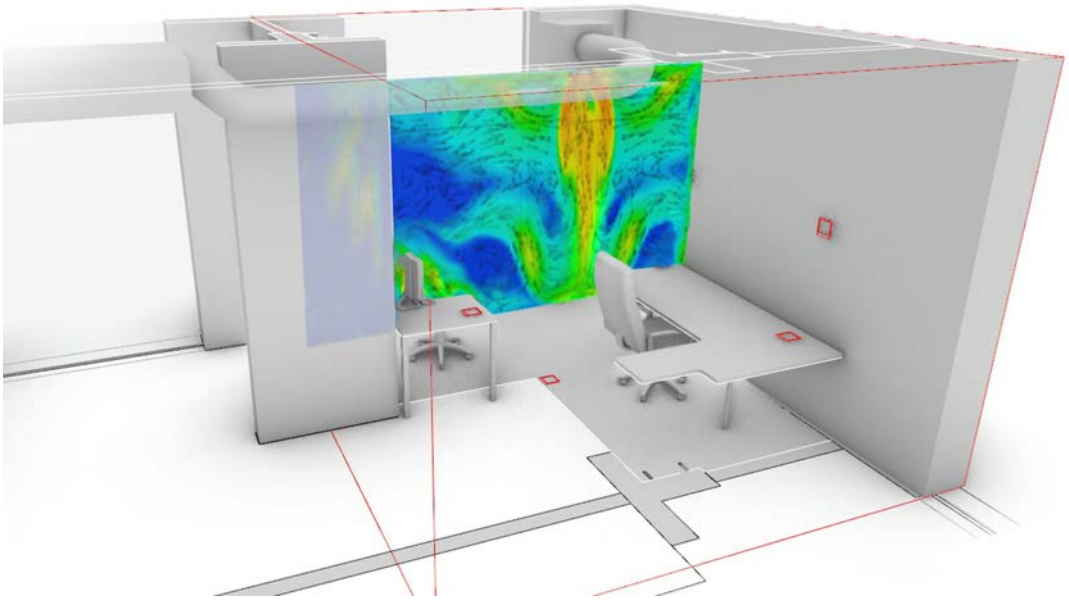


Figure 11. CFD analysis of airflow In the office test space

The location of the probiotic installation within the office space was determined following airflow simulations of the space, using CFD simulations. The model factored

for an overhead, centrally located HVAC system and the final position of the tiles aimed to make use of the airflows this system created as a way to potentially aid the spread of the microbes around the office.

Based on the size of the office and CFD simulation of the airflow, a series of twenty tiles were designed and tiled in portrait fashion (four across and five down), creating a probiotic surface of 60 cm × 75 cm. The tiles were fabricated according to the the meso-scale methodology of the previous phase, and sterilised using an autoclave. The tiles were then seeded with soil microbes and fixed to a plywood backboard, using standard interior tile adhesive for final fixing to the office wall.

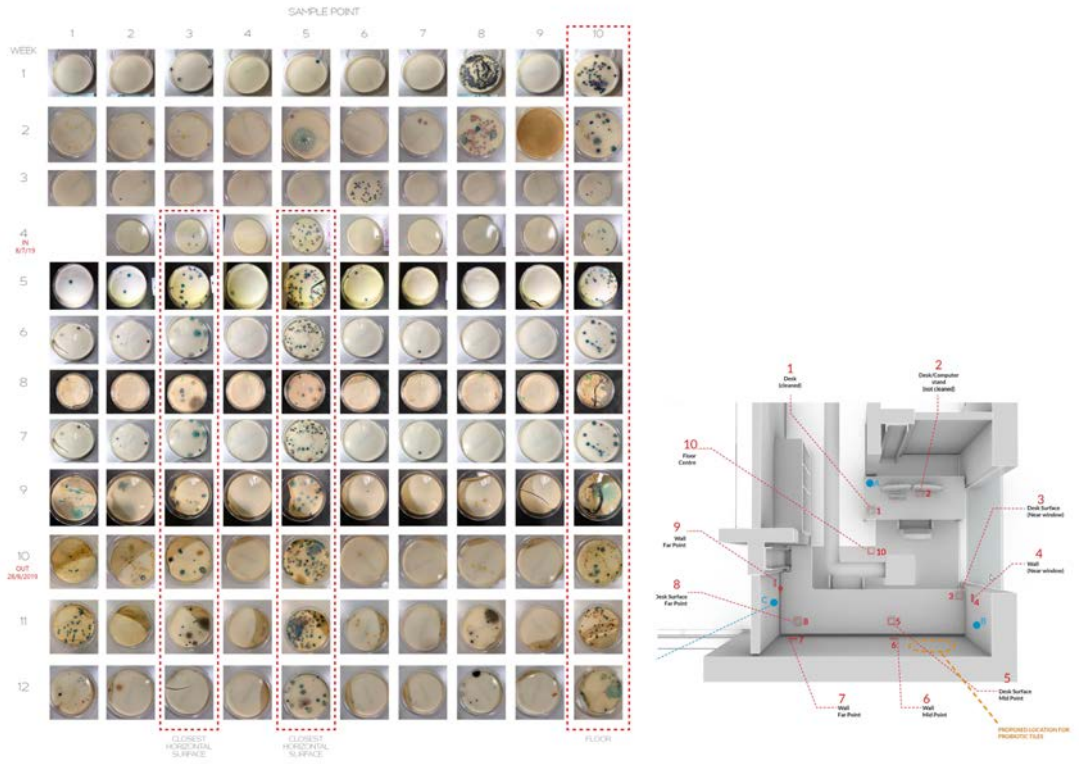


Figure 12. Culture plates taken from the office space over the 12 week study

The office space was sampled at weekly timepoints with cotton bud swabs taken from each of the ten selected locations. Sample points on surfaces were demarcated using acrylic grids to ensure repeatable location sampling. The sampling study ran over nine weeks covering a timespan including three weeks before, three weeks during, and three weeks after the installation and subsequent removal of the probiotic material prototypes. Samples taken were then processed and analysed in the laboratory to understand total bacterial diversity, using plate culturing and high-throughput sequencing methodologies.

The results from the culturing plates (Fig. 13) support the hypothesis that a probiotic intervention is able to change the microbiome of a space by increasing the presence of environmental microbes within a certain proximity of the intervention. The extent of this in relation to the full indoor microbiome of the space will come from statistical analysis of the microbial loads and diversities measured. This data is currently on hold due to the pandemic, but will be published separately in an appropriate journal.

The results here are discussed in relation to the culturing plates as a clearer way of describing the findings, compared to statistical graphs and sequencing data. They appear to support the hypothesis evidenced by the recognisable changes in microbial presence in certain locations. Clear differences are observed following the week 3 installation date for swab sites 3 and 5. Since these are the two sites in closest proximity to the intervention, the probiotic tiles appear to have an effect, but only up to a certain distance, approximately within the radius of 1 m. The spreading is probably the result of the airflows from the HVAC system in the space. This raises important questions in relation to how many microbes or how much wall coverage would be needed to fully re-contaminate an indoor space with environmental microbes.

Conclusion

The sanatorium movement of the early nineteenth century may have been the last example of architecture intended as a direct place of health. Developed in relation to the tuberculosis spread at the time, the movement was mostly unsuccessful in its attempts to cure the disease, as it was fundamentally based on the miasma theory of the time before tuberculosis was properly understood as a microbial infection. Developments in medical thought, health, and architecture share a history of exchanges and it may again be time for architecture to shift in line with contemporary understandings of health in relation to the human and built environment microbiome. While health remained a key driver of modernism, the emergence and widespread success of antibiotics in the mid-twentieth century in relation to infectious disease allowed architects to move away from health as a fundamental part of design. Their focus instead shifted to other areas of comfort, articulated around a climatic and sustainability-driven discourse. Yet, it appears that the overreliance on antibiotic approaches to microbes and architecture in cities has unwittingly resulted in the emergence of new pathologies, including inflammatory and chronic diseases. While they are less quick and fatal than infectious diseases, these are much slower illnesses that decrease the long-term quality of one’s life.

The probiotic design approach shown here is a first tiny step towards a much larger picture which shows that we need to find ways to massively reintroduce diverse environmental microbial communities into urban environments. While this needs to happen both at the urban scale, with planners and political interaction, this work serves as a proof of concept that this issue can be partly addressed through the material condition of buildings.

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Figure captions

Figure 1. A probiotic indoor microbiome approach using a multi-scalar design process, © Richard Beckett

Figure 2. A range of materials tested for material-microbe viability, © Richard Beckett

Figure 3. Testing material-microbial viability through regermination, © Richard Beckett

Figure 4. Results from stage one testing, © Richard Beckett

Figure 5. Microbiological methodology to determine microbial survival via cell counting, © Richard Beckett

Figure 6. Evidence of biofilm and spore formation in the material matrix, and subsequent regermination after one month, © Richard Beckett

Figure 7. Three geometrical typologies from left to right: pitted geometries; branching geometries; and creviced geometries, © Richard Beckett

Figure 8. Pitted geometries were developed using CFD simulations for a tiling family of four types, © Richard Beckett

Figure 9. Tile fabrication testing using two-part rubber moulds made from 3D-printed positives; multi-material casts were then produced with zones of porous and non-porous materiality, © Richard Beckett

Figure 10. Surface detail of probiotic tiles showing the textural, porous surface of the probiotic zone, © Richard Beckett

Figure 11. A 10 × 4 arrangement of ceramic tiles, designed with spatially programmed probiotic zones. These tiles were installed as an exhibition of the work at The Barbican in London as part of the Life Rewired festival in 2019, © Richard Beckett

Figure 12. CFD analysis of airflow in the office test space, © Richard Beckett

Figure 13. Culture plates taken from the office space over the twelve-week study, © Richard Beckett

Notes and references

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