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Turbulent Casting:

Bacterial Expression in Mineralized Structures.

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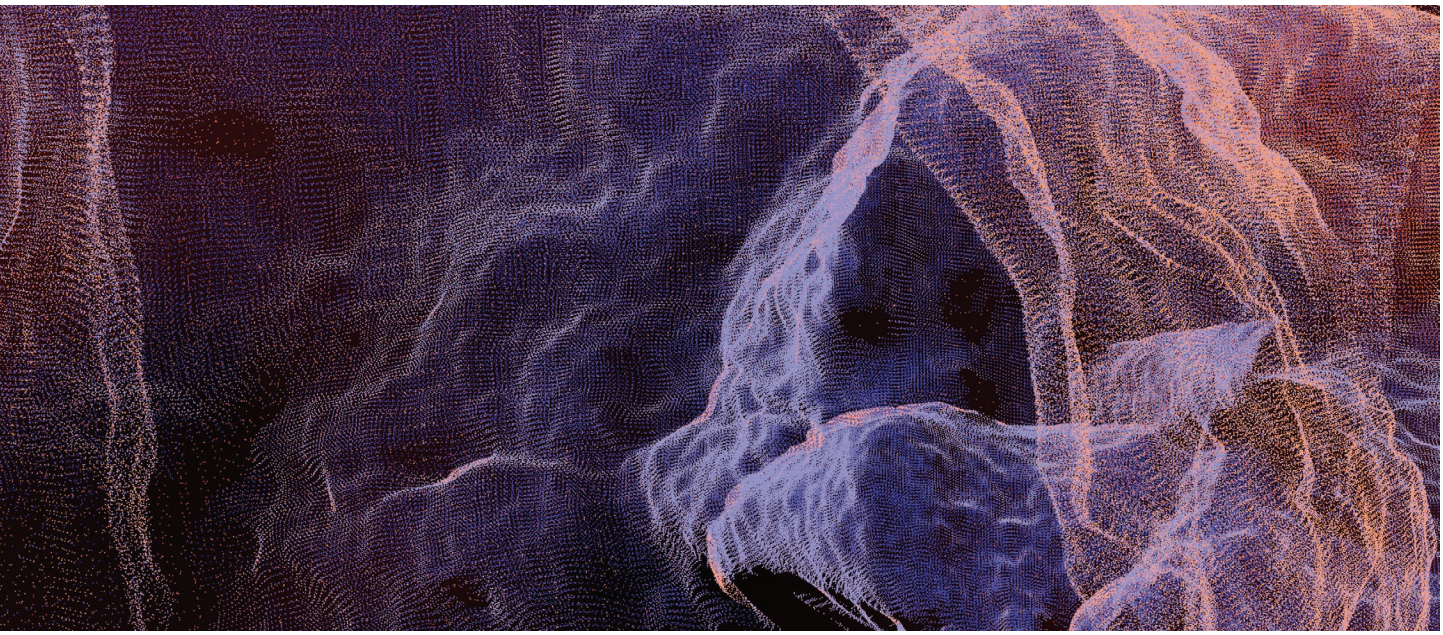

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ABSTRACT

There has been a growing interest in living materials and fabrication processes including the use of bacteria, algae, fungi and yeast to offer sustainable alternatives to industrial materials synthesis. Microbially induced calcium carbonate precipitation (MICP) is a biomineralization process that has been widely researched to solve engineering problems such as concrete cracking and strengthen soils. MICP can also be used as an alternative to cement in the fabrication of building materials and, because of the unique process of living fabrication, if we see bacteria as our design collaborators new types of fabrication and process may be possible. The process of biomineralization is inherently different from traditional fabrication processes that use casting or molding. Its properties are influenced by the active bacterial processes that are connected to the casting environment. Understanding and working with interrelated factors enables a novel casting approach and the exploration of a range of form types and materials of variable consistencies and structure.

We report an experiment with partial control of mineralization through the design of different experimental vessels to direct and influence the cementation process of sand. In order to capture the form of the calcification in these experiments, we have analyzed the results using three-dimensional imaging and a technique which excavates the most friable material from the cast in stages. The resulting scans are used to reconstruct the cementation timeline. This reveals a hidden fabrication/growth process. These experiments offer a different perspective on form finding in material fabrication.

1 Tectonic landscape in the point cloud construction resulting from the 3D scanning process of a biomineralized form.

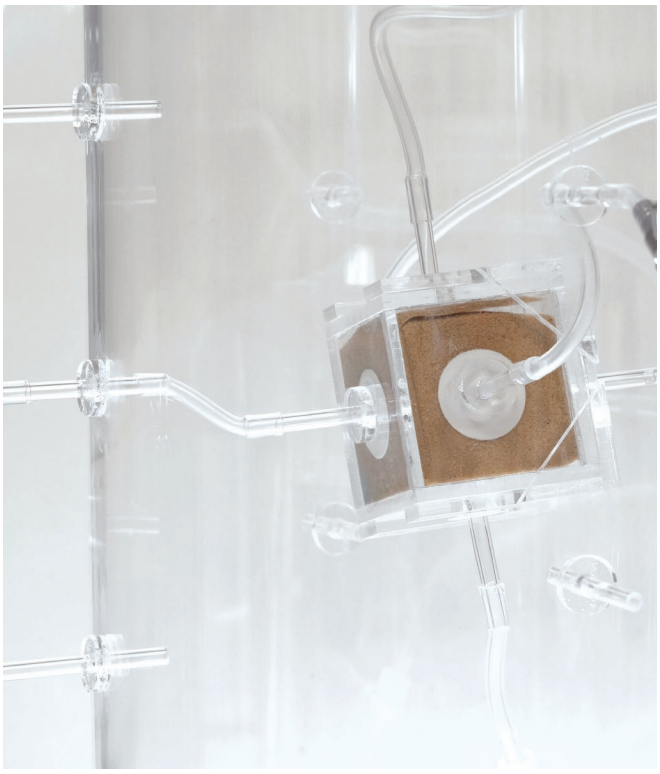
INTRODUCTION

Industrial-scale materials manufacture often creates an enormous strain on our environment. In construction, concrete is the most widely used material in the world and a significant contributor of the world's carbon dioxide emissions, producing more than 4 billion tons every year (Lehne and Preston 2018).

Nature, however, is the producer of hard calcareous materials in various forms through biological processes that have evolved over millions of years with relatively little energy expenditure. While biologically produced materials, such as limestone, are common in the built environment, we tend to make use of biological materials after the organisms which created them are dead. The possibility of using living cells through biological processes offers the opportunity of replacing traditional types of fabrication and assembly with guided growth (Zolotovsky, Gazit, and Oritz 2017) and new collaborations between non-human/biological processes in fabrication (Camere and Karana 2018). These processes entail the biological processing of elements from the environment such as carbon, water and sources of energy to form mineralized structures (Mann 2001). Microbial calcium carbonate precipitation (MICP) is efficient in constructing hard calcareous materials at room temperatures and pressures. The process of MICP requires active bacteria, a passive aggregate, and a catalyst for inducing the mineralization process to create a binder for the aggregate material.

MICP has already been demonstrated in building construction including, for example the inclusion of microbial spores in concrete to enable self-healing of concrete cracks (Jonkers et al. 2010) and for the creation of an MICP based cement to create bricks or other natural stone-like building elements (Dosier 2016; BioMason 2020). While having the potential to transform traditional construction the use of MICP as a novel fabrication method has not been substantially explored. There has also been extensive design speculation on the use of MICP in Architecture including, for example, the injection of bacterial solutions into sand dunes to stabilize them and creation structures for human habitation (Larsson 2010) and as a method for constructing furniture using similar approaches (Hussey 2014). Projects such as these, rarely go beyond design concepts as the process of MICP based material casting is not straightforward to perform.

While MICP based materials can be produced in similar ways to more traditional cementitious materials, using microbes as active agents in MICP offers opportunities for form making and material construction which are unique



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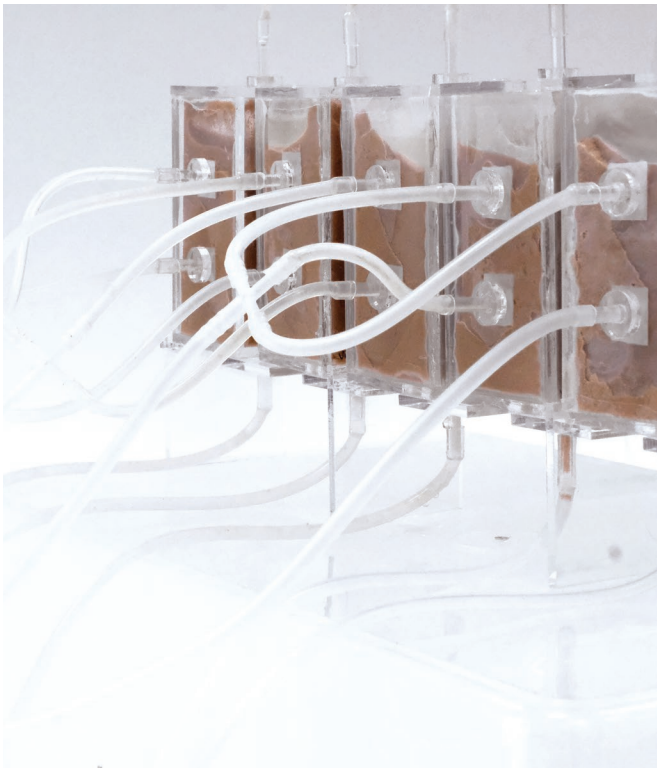

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2 Prototype 2, casting vessel showing connection between the suspended mold and the bioreactor.

3 Prototype 3, casting volumes showing the series of molds set up.

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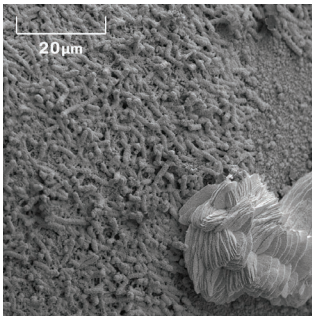

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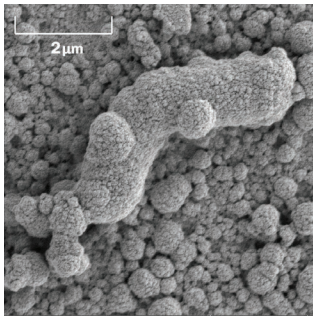

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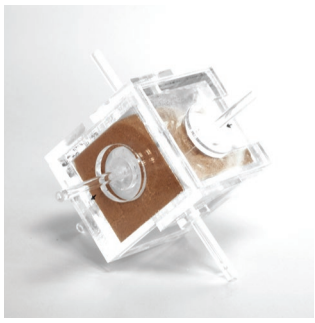

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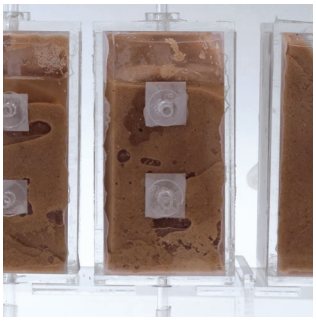

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4 SEM of a calcified Sporosarcina pasteurii in agarose hydrogel.

5 SEM of a calcified Sporosarcina pasteurii in agarose hydrogel.

6 Prototype 1, casting volume.

7 Prototype 2, casting volume.

8 Prototype 3 casting volumes.

9 Prototype 4, casting volumes.

to the medium. However, to move beyond the state of the art requires repeatable hands-on experiments based on the production of meaningful material samples with the potential to scale up. In this paper, we present ongoing research into MICP in order to inform design engagement with a living organism in the creation of a mineralized material.

This paper focuses on the correlation between the fluid-solid interface that influences the flow of liquid through porous media (Wood, He, and Apte 2020). It explores how this phenomenon effects the fabrication process of bacterial-induced biomineralization and frames the living bacteria as co-designers in a construction system. It reveals a process of biological computation where the fabrication shows sensitivities to inputs which lead to different material outputs. Through this exploration, it will be shown that the MICP process is inherently different from traditional fabrication processes using, for example, casting and molding. We present initial studies of a casting process involving MICP in the sand which show the complex network of interactions essential in the control of biomineralization. The experiments focus firstly on the biological function of the bacteria in the precipitation of calcium carbonate and the optimal environment needed to support them. The project experiments with the design of experimental vessels to influence the cementation process. Finally, in order to capture the calcification in these experiments, the cementation pattern formation was analyzed using a three-dimensional scanning (see Fig.1) and a novel 'excavation technique' which allows the dynamic build-up of the cementation process to be visualized.

METHODS

MICP is a biomineralization process where the precipitation of calcium carbonate occurs in response to microbial activity in specific environmental conditions. In our experiments, we incubated bacterium *Sporosarcina pasteurii* in various physical and chemical conditions for growth and mineral precipitation.

The bacterial cells use biological and chemical processes to form inorganic carbonate crystals by creating a microenvironment where hydrolysis of urea and the increase in pH around the cell induce the production of calcium carbonate ($CaCO_3$) crystals. The bacteria act as a nucleation site for $CaCO_3$ precipitation (see Fig. 4-5). Mineral crystals are not produced by the bacteria but, rather, induced in response to an enzyme-initiated change in environmental pH. Many factors can affect this process, including the growth rate of the bacteria, availability of oxygen and nutrients as well as the chemical components. These are interdependent parameters that can affect the construction of a biomineralized material at different scales.

For this casting process, we looked at these variables as tuning parameters to experiment with form-finding in the fabrication of cemented material. The parameters that we explore in this paper are:

- **The microbial factor:** as the biological and chemical activity of the bacteria in the mineral production of calcium carbonate crystals.
- **The granular factor:** through sand shape and size that dictates the structural control and binding possibilities.
- **The casting factor:** how the mold influences spatial control of the elements contained inside and how fluid matter is applied.

- **The fluid factor:** the chemical control of a mixture of nutrients, urea and calcium chloride, to apply to the system (the cementation media).

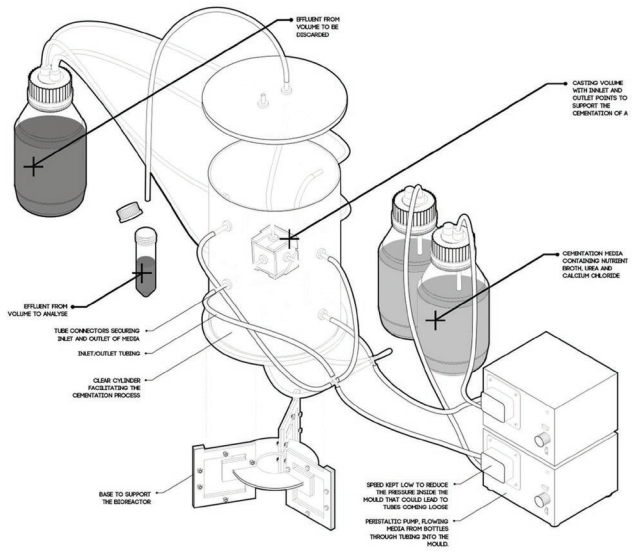
Although the tuning of each of these factors will affect the formation of a biomineralized structure the biological elements of the bacterium are not being altered in a direct way, i.e., the bacterium was not genetically modified. The bacterium is, however, affected by the environment and changes in those factors can speed up or slow down their reaction time, spatial distribution, reproduction and survival.

Apparatus

In this study, prototype casting vessels were developed as part formwork, and part bioreactors in order to explore the different influences, such as casting and fluid factors, and direct the cementation process. These prototypes facilitate the biocementation process by containing sand, fine enough to allow the bacteria to move between the grains, and by pumping the nutrient-rich liquid media with the substrate chemicals, urea and calcium chloride, to facilitate the essential bacterial activity to induce mineral crystal formation.

The casting vessels are constructed using digital fabrication, from clear acrylic that allows visual observation, and silicon tubing. The tubing allows injection of cementation liquid into the mold using peristaltic pumps at certain intervals per day as well as letting effluent waste liquid out. The acrylic mold is lined with acetate film to allow for easy release from the mold and filter paper in order to contain the sand and keep it from flowing back into the inlet tubes. The vessel is first filled with sand that is injected with a bacterial culture which has been incubated overnight in a shaking incubator at 30°C, to allow the bacterial cells to grow and attach to the sand grains. After this stage, the volume is connected to the pumps via tubing and the cementation solution is injected to initiate the mineralization process. The fresh solution is pumped through the vessel at 3-4 hour intervals over 7-10 days. In this process, pH levels are monitored from the effluent each day. It is important to maintain a pH of as close to 9 in order to enable the bacterial cells to induce crystal formation. If the pH starts dropping below 9 it is a good indicator that the cell density has dropped, or the bacteria are not receiving the nutrients they need. The rate of precipitation induced by the bacterial cells can also be increased up by increasing the amount of calcium chloride in the liquid medium.

Each type of vessel (see Fig. 6-9) is designed to deliver the liquid media into the sand volume through a different flow



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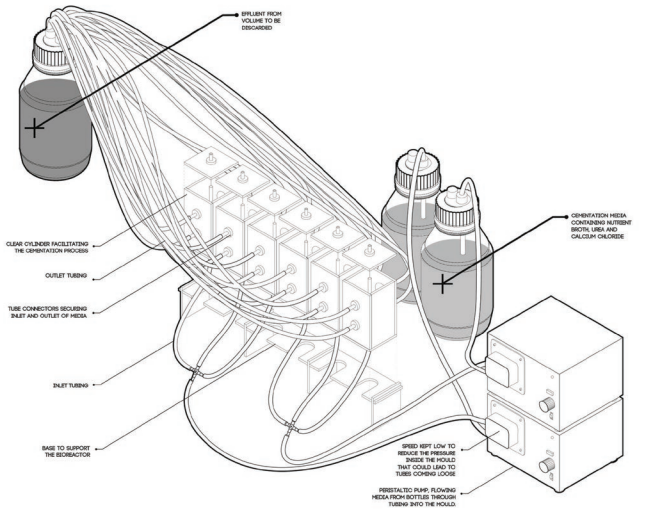

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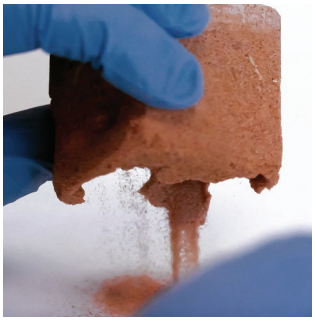
10 Exploded view of prototype 2 showing running setup and the connections with inlet media and effluent through peristaltic pumps.

11 Exploded view of prototype 3 showing running setup and the connections with inlet media and effluent through peristaltic pumps.

strategy in order to see the different effects they can have on the cement formation. Initial experimentation followed known cementation procedures using plastic syringes (Whiffin 2004) to inject media upwards through a cylindrical volume (Fig. 6). This first prototype was used to experiment with:

- different sand sizes;
- the way the bacteria culture was mixed in the sand;



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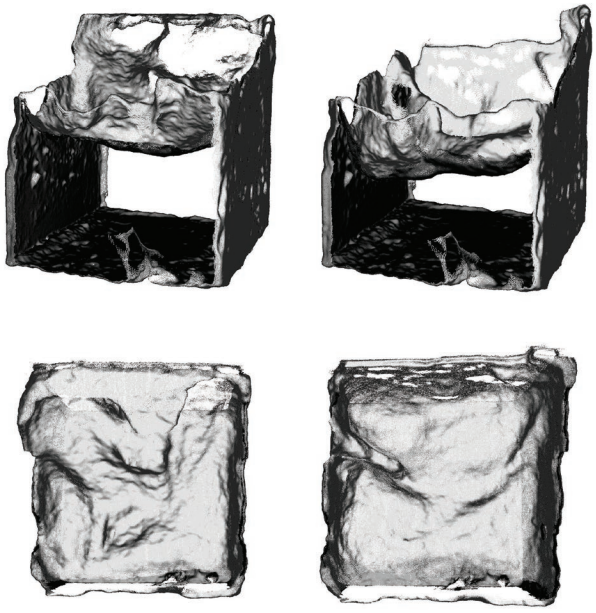

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12 Loose sand removed from internal parts of the sand cast from 2nd prototype.

13 Sand cast being brushed with gentle brush strokes as the looser sand is removed from a volume in the 3rd prototype.

14 Point cloud construction resulting from 3D scanning a sample. Visible flow patterns emerge from sand cast from the 2nd prototype, showing two stages of the erosion.

- if applying heat or extra oxygen in the nutrient media would make a significant difference in the cementation procedure;
- and how long the cementation reaction would take.

Once optimal conditions were established, we used sand of 150-300 µm, the bacterial culture was either poured or injected into the volume in a liquid medium and when the cementation treatments started, we kept the volume at room temperature (21-23°C). In these experimental vessels, due to their small size, at least six days were needed of cementation treatments to form a solid sample piece.

The second prototype vessel (see Fig. 2, 7, 10) has the shape of a cube of 5 x 5 cm that was with tube inserts on all sides. This setup allowed media not only to flow from below, as in the first vessel but through multiple injection points on all sides. This method eliminated the preferential flow that occurred when only pumped in one direction. Only three sides were selected for inflow of cementation media while the other three openings allowed effluent out, and this arrangement was changed every two days. This setup ran for ten days before the sample cube was removed and dried. Eliminating preferential flow by changing flow paths created a relatively homogeneously cemented cube shell.

The third prototype (see Fig. 3, 8, 11) was made of cuboids of 5 x 10 cm in which the cementation media was injected through the center of a volume through a perforated tube connected in the middle of the cast with effluent media pushed out through the outlets positioned at the sides. This was to ensure that the internal parts of the sand volume were supplied with sufficient amounts of nutrients and reactant chemicals for the bacteria where previous prototypes prevented this access by cementing and blocking off where the inflow reached the volume. This prototype was set up as six identical volumes stacked together. Each cast was connected to a pump that supplied cementation media to all volumes at the same time. This setup ran for a total of twelve days. Here, every other day during this treatment, one volume was disconnected from the stack, opened and dried. This was to create a series of mineral formations where we are able to track the stages of cementation.

The fourth prototype (see Fig. 9) was made of acrylic tubes 10 cm diameter and 2.5 cm in height and, as with the previous version, the vessel was designed to be injected from the inside. Openings were cut into a central plastic piece that was connected with tubing to the pumps and effluent points positioned on the exterior of the acrylic tube. This prototype was set up as three identical volumes stacked side by side, which ran for six days. What distinguished it from the previous setup was the ability to rotate the whole vessel in order to eliminate the occurrence of preferential flow or static air pockets in specific parts of the volume. The volumes were then opened and dried and analyzed alongside the other prototypes.

3D Scanning

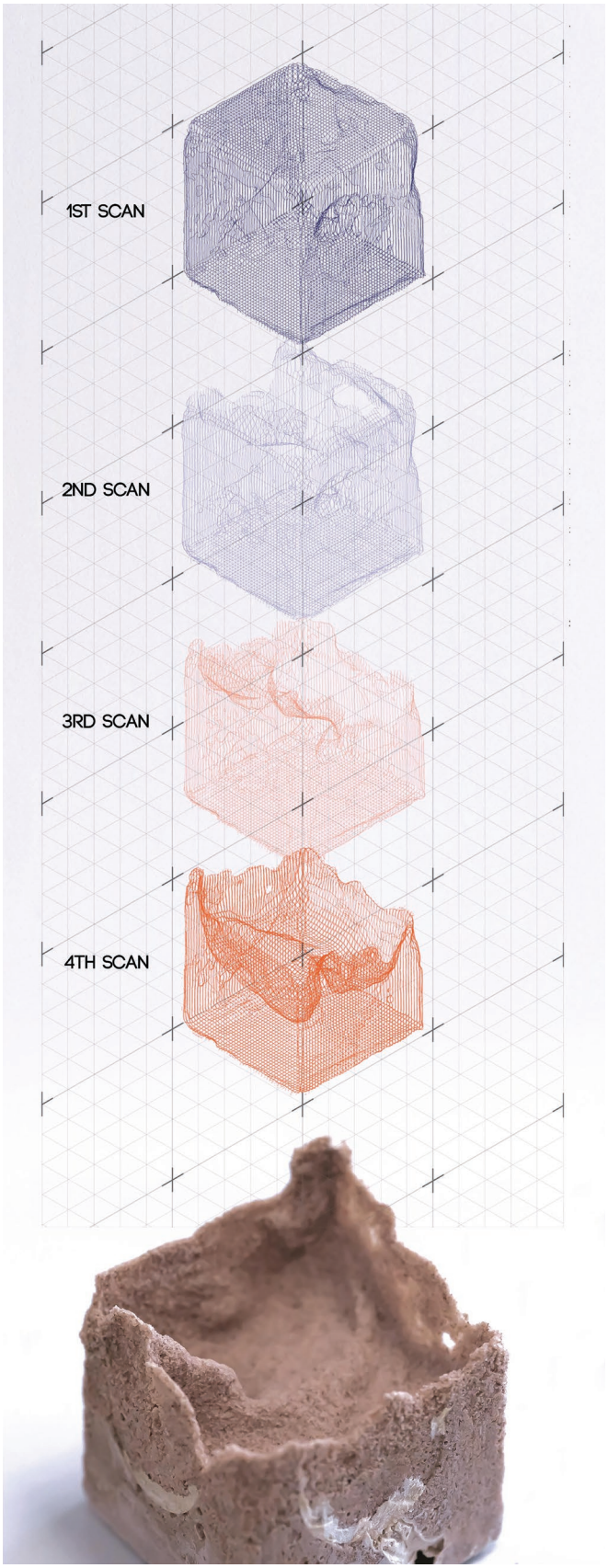
For each of these different fluid injection methods, 3D scanning was used to explore the biomineral formation that occurs within the volume (see Fig. 1,17), something that is not visible during the process. Scanning at different times in the process can give us a glimpse into the patterns that form at different stages in the cementation process. The 3D scans were processed using EinScan-SE, a digital

scanning program that uses programmed light patterns (Salvi et al. 2010). Between 30 to 100 scans were used to reconstruct the samples, producing a digital representation of the cemented objects in a 3-dimensional point cloud. Similar scanning techniques have been used before in detecting and analyzing biological growth (Sollazzo, Baseta, and Chronis 2016). The resulting casts from the prototypes were 3D scanned in a sequence from the moment they were taken out from the casts and in steps, as the sand grains were gradually brushed using a small spatula and brush where the material was more friable (see Fig. 12-13). This excavation process allows us to trace back the cementing formation and reveal the different densities the material is able to form through time. These produced between 3-4 stages in the point cloud model depending on how friable the material was. Once these point models were generated, they were exported to Rhinoceros 3D (see Fig. 14) and sliced to create topological sections (see Fig. 15) that highlighted the transformation of the cast. These resulting scans can be seen as a timeline of the stages of cementation and allow us to visualize material changes over time.

RESULTS & DISCUSSION

The 3D scans from the excavation process reveal patterns of flow in both space and time. This was especially apparent in the case of the third prototype, where excavation revealed that the initial solidification occurred at the top of the volume (see Fig. 16). As days passed, the cementation spreads to the rest of the volume from the close to the central injection tube outwards, eventually cementing the bottom of the volume.

The material samples explored in this study reveal that the way in which the flow is channelled in the sand constitutes a key factor in how the cemented form develops in the volume. The clear visualization resulting from the 3D scans highlights paths of the preferential flow of reactant media and its concentration in specific parts of the vessel. This preferential flow results in areas of a higher density of calcium carbonate precipitation. As bacteria mineralize, the flow of liquid changes its path, always finding the path of least resistance around solid formations and triggering crystallization in the remaining areas of the cast. As the liquid flows within the volume, it meets a shifting environment, whose topology is determined by the activity of the bacteria and resulting cemented formations. The natural patterning of the precipitate mostly depends on the distribution of bacteria cells and on the concentration of the reactants that trigger biomineralization activity. The various degrees of friability in the cemented material indicate that the flow meets the sand at different speeds through the volume. The speed of the flow decreases due to



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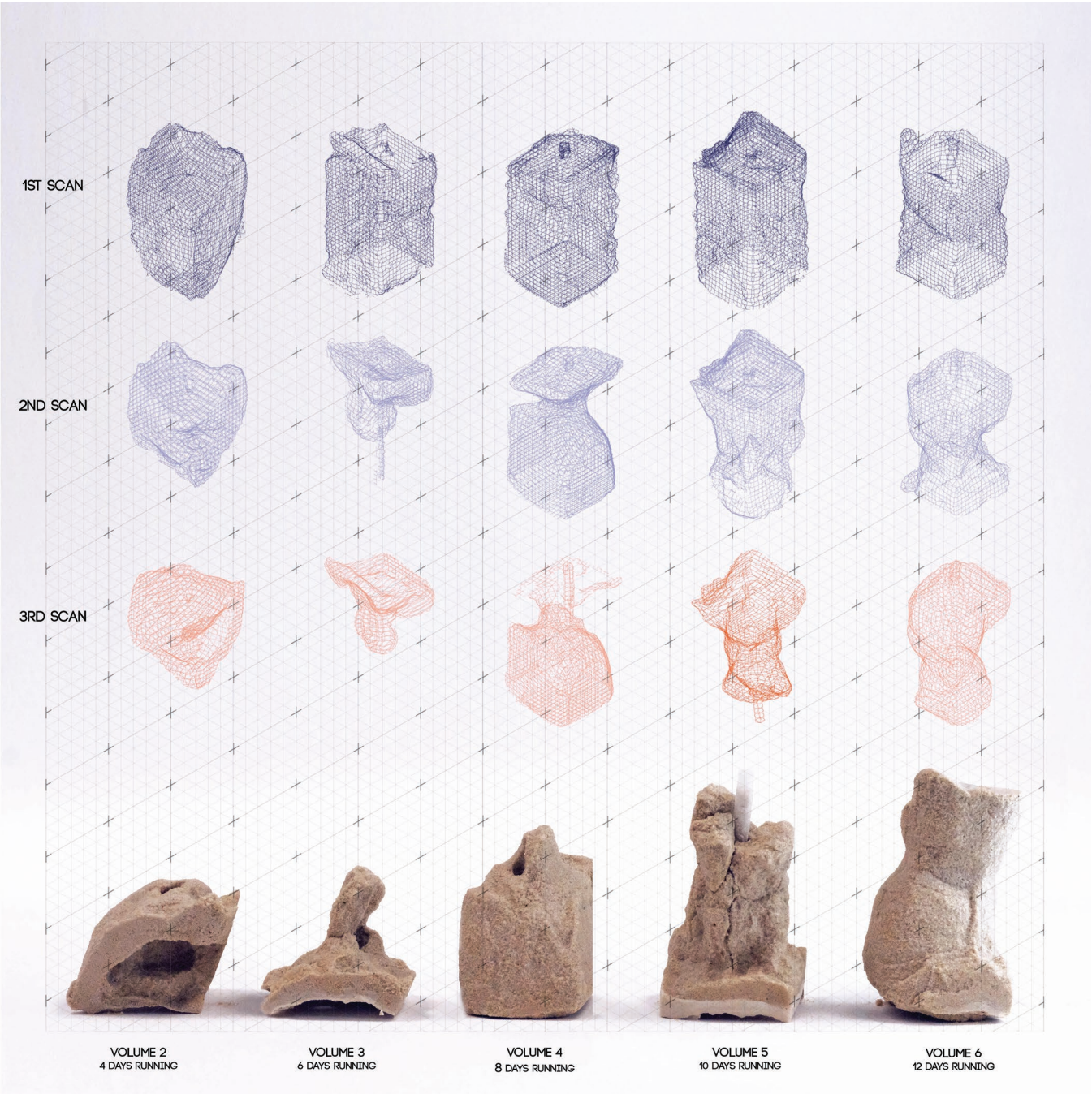
15 Topological sections of the time line stages of a sample from the second prototype.



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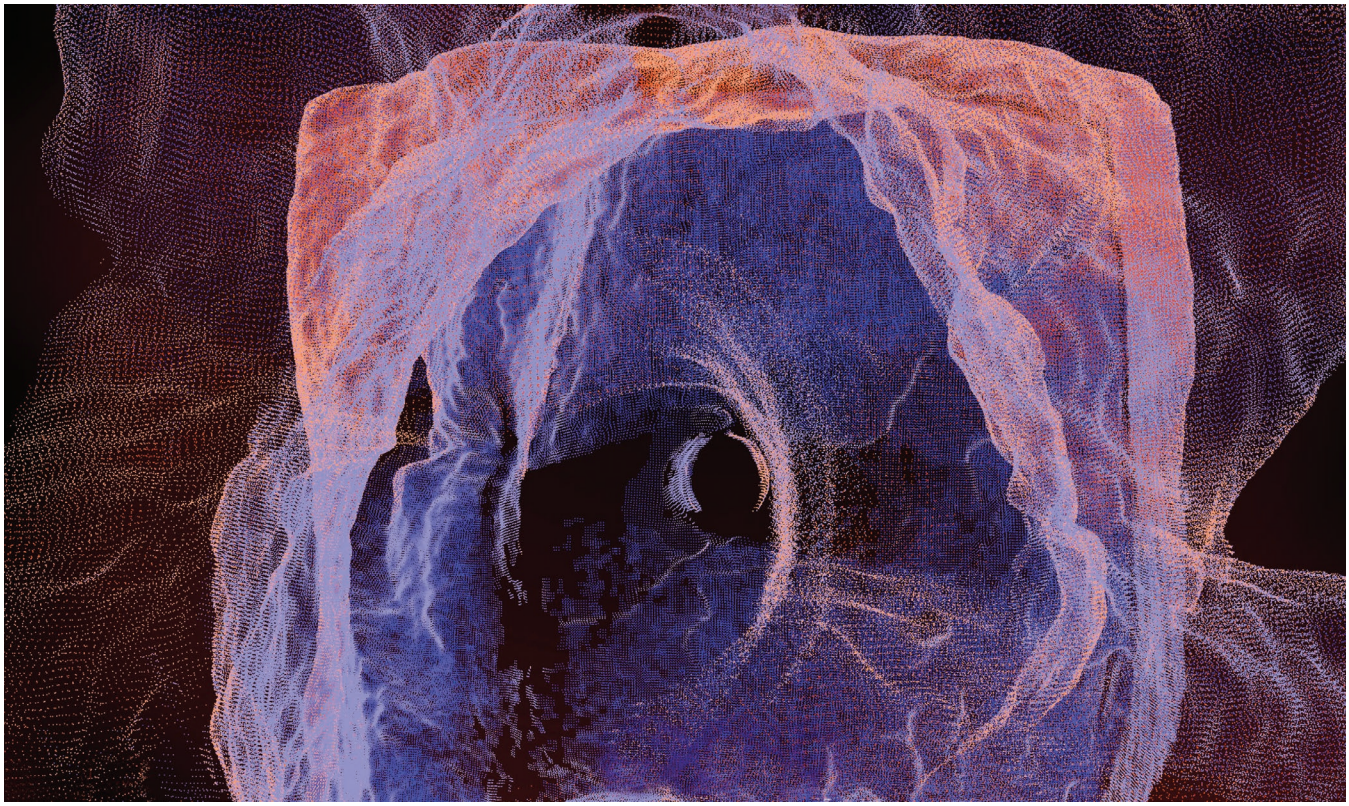
16 Topological sections of the time line stages of the different samples from the third prototype. Showing the different formation from a 4 day treatment to 12 days of cementation treatment with the resulting casts standing (in revers) on the top cementation.

blocking caused by precipitation (Saad et al. 2019). In other circumstances, a decreasing dimension of the flow channel would correspond an increase in pressure, and therefore in speed, but in this case, the internal circulation channels are still filled with sand the pressure is transmitted to the pump where it is forced to slow down. Where cement begins to block areas of flow, the reactant will have to circle in order to reach the non-cemented areas. As the speed of the flow diminishes, the bacteria left in these areas are starved of nutrients and start to die off. When, and if, the flow reaches these areas, the crystal formation will consequently be minor, causing the sand to not be bound as tightly and be more friable.

CONCLUSION

These results demonstrate the unique complexity of a casting process which involves living systems. The bespoke vessels allow the exploration of biomineral fabrication, and our experiments show that, while containing consistent features, each cast is unique and represents a story



17 Tectonic form in the point cloud construction resulting from the 3D scanning process from the third prototype in volume 5. Seen from the bottom of the shape looking up though the gap left by the injection tube.

of the living dynamic processes of the casting process. The results are not a homogenous material but graded and sculpted volumes. Through these experiments and 3D imaging, we recognize that the control of the designer, through the casting process, is only partial. By controlling environmental factors through the vessel, we generate optimal conditions for the bacteria to live and for cementation to occur, but we cannot fully control the outcomes. The biomineralization process, as applied in architecture, is a particularly complex and radically different medium to fabricate with compared to traditional non-living materials. The mineralization process is not produced by the organism itself but is an induced chemical reaction in the environment caused by the bacteria. Therefore, these experiments encourage speculation regarding how this bacterial process is expressed and formed within aggregates such as sand through the fluid delivery of a catalyzing agent.

Although only preliminary work is presented in this paper, this technique of capturing the formation in reverse time, by taking layer by layer off the sample and scanning each step, offers an exploration into form finding and challenges the idea that, in casting, the material takes the form of the cast. The process reveals heterogeneity which is hidden in the casting process but may offer new architectural aesthetic and materials which reveal their fabrication process and the imprints of life. Further avenues of exploration would be to build up further data from these experiments that could be used to simulate the process and to scale up the casts for architectural applications in the built environment.

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IMAGE CREDITS

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Dr Meng Zhang is an Associate Professor in Microbial Biotechnology at Northumbria University. Her PhD was in Proteomics studies of streptococcal pathogens, and she has worked on Biocatalysis as a postdoctoral researcher for eight years. More recently, Meng has established new interdisciplinary

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Dr Beate Christgen is a Senior Research Associate in the School of Natural and Environmental Sciences at Newcastle University. With a background in applied chemistry, advanced wastewater treatment and environmental molecular microbiology, she studies changes in natural and engineered microbial systems and how to exploit these systems for a sustainable future. Her research includes investigations of bioelectrochemical systems for energy and product generation from wastewater, the impact and mitigation of the biological methane cycle in the Arctic on climate change, antibiotic resistance gene abundance and dissemination through wastewater and in terrestrial ecosystems, and microbial induced calcium carbonate precipitation in hydrogels.