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(Re)thinking Resilience

Robotic additive manufacturing of lichen composites for air quality monitoring

Digital fabrication of responsive and resilient material systems from lichens

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ABSTRACT: In the light of an unprecedented climate emergency, there is an urgent need to reconsider the materials we use for the construction of our buildings and to explore alternative material systems that are abundant, easy to source, and exhibit increased climate resilience and adaptability. Materials with these attributes have been developed by living systems. We propose the development of hybrid materials from living lichens and abiotic components for air pollutant sensing, using a 3D digitally driven paste delivery system. Lichens are excellent indicators of air pollutants, they present resilience to extreme environmental conditions and are abundant, can self-grow and self-regulate. The lichen species Flovoparmelia caperata, Parmotrema perlatum and Xanthoria parietina were used for the production of the samples. We employed a robust photographic recording system that allowed the morphological monitoring of the samples. We assessed colonisation tendencies and performed statistical analysis of changes in pigmentation distribution to understand substrate-lichen correlations and the metabolic activity change over time. Our findings demonstrated that three combinations of lichen species-substrates presented optimal integration and were selected as most suitable for 3D printing. We also report that one sample demonstrated excellent colonisation behaviour and less changes in its metabolic activity over time.
KEYWORDS: hybrid living materials, lichens, robotic 3D printing, biosensing, sustainability

1. INTRODUCTION

The building industry accounts for one of the largest negative impacts on the planet, significantly contributing to the depletion of natural resources and raw materials. In addition to this, we are already experiencing pressure on living conditions and an increase in damage to buildings from extreme weather events, attributed to climate change [1]. In the light of this unprecedented climate emergency, there is an urgent need to reconsider the materials we use for the construction of our buildings, their production and assembly processes. We therefore need to explore alternative material systems and fabrication processes that allow the use of materials that are not only abundant in our planet, readily available and easy to source, but also exhibit increased climate resilience and adaptability, mitigating the impact of extreme climate change events and responding to the ever-changing environmental conditions.

Materials with responsive capabilities have already been developed by living systems over the last 4.5 billion years, as they have created biological structures for the modification of their environment and survival [2]. Natural biological systems are capable of sensing environmental conditions and responding to them on multiple scales [3]. Living systems possess distinctive features that endow them with intrinsic advantages for the manufacture of responsive materials such as their ability to grow autonomously into structured bulk materials without human effort, to self-regenerate and degrade the components that they make, thereby possessing a sustainable life cycle for the entire material and to provide complex responses to environmental stimuli [4]. In addition to the above features, they exhibit living attributes such as self-growth, self-regulation, self-repair and self-replication, making them excellent candidates for the development of materials with reduced environmental impact as compared to conventional materials. The above exceptional properties and capabilities make living materials attractive as functional components for the development of responsive materials.

An emerging area of materials that has attracted interest in the scientific community is Living Hybrid Materials. This class of materials can be defined as materials composed of living cells that form or assemble the material itself, or modulate the functional performance of the material in some manner [2]. Living hybrid materials contain both living organisms and abiotic components. They retain the living attributes while the incorporated abiotic materials enhance the material structure and performance such as responsive and sensing functionalities [4]. Living hybrid materials with

sensing and information processing capabilities usually incorporate a substrate, and functional or active layers [5]. The functional layers include the active elements where the main activity takes place while the substrate is a solid substance upon which the functional layers are deposited [6, 7].

This research project aims to contribute to an emerging and growing body of work that recognises the potential of living cells as functional layers for the development of materials with responsive capabilities such as sensing external stimuli. We propose the development of hybrid living materials from lichens, using a novel robotic additive manufacturing process for paste 3D printing of the lichen substrate matrix.

Lichens are symbiotic organisms, usually composed of a fungal partner and one or more photosynthetic partners [8]. They are found on trees, rocks and in soils [9] and are perennial, resilient and able to live for many years in extreme conditions [10,11]. They are abundant, providing easy accessibility, they can self-grow, self-repair and they are self-regulating. Unlike plants, lichens lack vascular organs to directly control their water loss or uptake [12], their water content equilibrates with atmospheric conditions and as a result, lichens range between desiccated and water-saturated states on a daily basis throughout much of their lifetime [13]. Lichens can survive in harsher environments compared to their constituting fungi and algae as individual living microorganisms due to passing nutrients and metabolites to each other which ensures better adaptability [14]. This astonishing capability to survive and remain living and healthy under extreme environmental changes, makes them promising candidates to be used as a sustainable alternative to conventional materials currently used in the building industry.

In addition to exhibiting resilience to extreme environmental conditions, lichens are excellent biomonitors for air pollutants. The use of lichens as biomonitors is attributed to their ability to respond to air pollutants at different levels, their slow growth rate, longevity as well as their ability to indicate the presence and the concentrations of these pollutants [15,16]. Although lichens have been used extensively in the field for the detection and monitoring of air pollutants such as metals and organic air pollutants, to our best knowledge, integration of lichens into substrates for the development of building material composites has not been reported yet. We therefore present a scoping study in which we investigate the integration of lichens into various substrate matrices using a digitally driven paste delivery system.

2. HYPOTHESIS AND OBJECTIVES

Drawing from the existing literature demonstrating lichen's ability to respond to extreme environmental changes as well as sense and report the presence of air pollutants, we hypothesise that it might be possible to 3D print lichen composites that can perform the above functions. Our aim is to develop living hybrid material systems from lichens that exhibit responsive capabilities when exposed to environmental pollutants, using a novel digital fabrication method of paste delivery.

The hypothesis is sought to be verified through the following objectives:

- To assess and establish protocols of lichen/substrate colonisation for the development of the hybrids.
-To 3D print substrates that present optimal colonisation behaviour using a digitally driven paste delivery system.
-To evaluate gas absorption rates of hybrid materials upon exposure to pollutants.
-To develop a framework for deploying lichen hybrid material systems.

3. METHODS

3.1 Experimental set up and material synthesis

The lichen species Flovoparmelia caperata, Parmotrema perlatum and Xanthoria parietina were collected from three tree trunks of an outdoor area with average pollution levels in Bristol, United Kingdom. For each of the species, the same tree trunk site was used for the collection. The rationale for this decision was that since the same site was used, it was most likely that the specific lichen species benefitted from the same secondary metabolites such as nutrients and water intake, environmental stress, moisture content and sun exposure, allowing the development of conclusions by excluding these parameters when performing morphological characterisation and statistical analysis.

Seventeen samples of different substrates- lichen species combinations were produced (Fig. 1). The substance choice and concentrations were based on an already established protocol of material mixture and concentrations used for 3D printing. The materials used were Cellulose Fibre Scarva, Methyl cellulose, Chitosan, Glycerin, Agar, Coffee grounds, Rice Starch, Sodium alginate, Hemp Shive and Clay (Smooth Terracotta). Table 1 shows the composition and quantities of the developed substrate samples.

Figure 1: Inoculated substrate samples. Flovoparmelia caperata, Parmotrema perlatum and Xanthoria parietina lichen species were used for the inoculation. *Photograph by Simon Regan 2023.*

The samples were kept at ambient room temperature in a hydroponic growing tent with the provision of artificial daylight (Fig. 2) and monitored every 2-3 days. Additional moisture was added manually as required with de-ionised water.

3.2 Pigmentation distribution

A photographic recording system incorporating a customised 3D printed base for the positioning of a camera at an angle and distance that allows the morphological monitoring and characterisation of the samples was established.

Table 1: Substances and concentrations used for the development of the substrate samples.

Images of each sample were taken for 6 consecutive weeks prior to the implementation of data analysis (Fig. 3). We performed colour distribution statistical analysis. The rationale behind the selection of this method is the described below

Figure 3: Recordings of sample 16 for 6 consecutive weeks. Pigmentation distribution changes have been analysed to understand correlations between lichen species and substrate composition.

The parameters that may affect the distribution of the lichen pigmentation over time can be attributed to the following factors: 1. Fungal partner pigments 2. Algal or cyanobacteria pigments 3. Secondary metabolites 4. Interaction of lichens with the substrate 5. Type of lichens 6. Environmental stress 7. Moisture content. Since the inoculation of the substrates was performed in controlled conditions, factors 3, 6 and 7 remain the same for all samples and therefore can be eliminated from the parameters taken into consideration. Changes to pigmentation distribution over time may therefore be attributed to the interaction of the lichens with the different substrates as well as the composition of the fungal and photosynthetic partners in each sample. Conclusions may lead to a better understanding of how lichens interact with the substrates and which lichen species- substrate combinations could lead to integration and adherence of the lichens to the substrate substances and therefore to self-assembly formation mechanisms. For the interpretation of the data set depicting pigmentation change over time, the data of the same lichen species samples were analysed and compared to allow the elimination of the impact of factor 5 on our possible conclusions. MATLAB was used for the analysis.

Figure 2: Hydroponic tent used for the lichen-substrate inoculation to allow controlled conditions of light, humidity and temperature for all samples. Photograph by Simon Regan 2023.

3.3 Robotic 3D printing test system

The Robotic 3D printing test system used in this research project consists of an UR collaborative Robotic Arm which is connected to an Arduino based micro controller hardware unit. This unit operates a stepper motor which is integrated within Poseidon open-source syringe pump fixture that facilitate the delivery of the paste, synchronised with the movement of the robotic arm. The Poseidon fixture

has been modified to fit the UR end-effector bracket and adapted to hold a 60ml syringe tightly. The syringe itself has been modified with a set of interchangeable set of nozzles 3D printed in 5, 8 and 10mm diameter.

The workflow of processing test prints starts with forms designed in a CAD software and saved in the STL format. The STL files are then sliced into 3D printing paths using the CURA software. These paths are saved as G code data, which can be interpreted by the integrated robot arm and paste delivery system. To facilitate the interpretation, the CURA G-code commands are loaded into the RobotDK software running on a laptop which is connected to the UR control box via an ethernet cable – enabling real time data flow. Data is relayed from the UR control box to an Arduino microcontroller which in turn send commands to stepper motor driver that controls movement of the stepper motor which pushes the plunger of the syringe and ultimately deliver the paste in amounts that are synchronised with the movement of the robotic arm (Fig. 4).

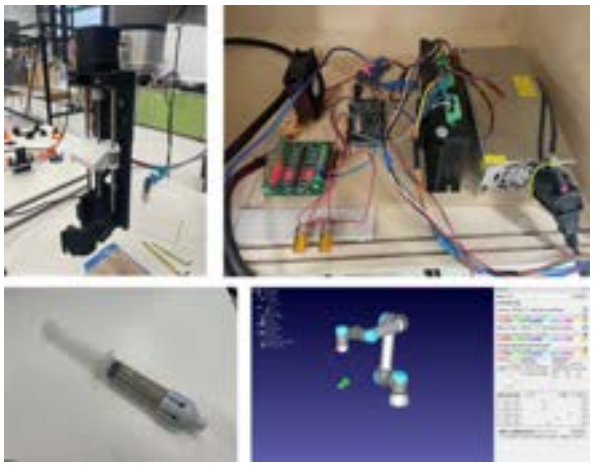

Figure 4: Images of the development of the robotic 3D printing test system. The system consists of an UR collaborative Robotic Arm connected to an Arduino based micro controller hardware unit using a syringe paste delivery printing process.

3. RESULTS

Samples 1, 2 and 11 presented optimal colonisation and the lichen species were fully integrated with the substrate, forming a hybrid material from the fusion of the living and synthetic particles. By optimal colonisation, we refer to the binding and attachment of the lichen species to the substrate in such a manner that the components are coalesced and cannot be separated.

It is worth noting that for the image classification via statistical analysis of changes in pigmentation distribution implemented in the analysis below, the red, green and blue mean values are the average values of the red, green and blue channels of the image pixels. In regard to the species Xanthoria parietina and for the course of 6 weeks, sample 2 presented the highest red and green mean values of 177.9 and 170.9 with standard deviation values of 49.4 and 55.0, skewness of 2.52 and 2.47 and kurtosis 8.97 and 8.55. This indicates that the sample presents leptokurtic distribution and the kurtosis is therefore positive. Additionally, the skewness is negative. On the other hand, sample 1 showed the lowest red and green mean values of 157.2 and 150.2, with 70.3 and 76.2 standard deviation values, skewness of 2.56 and 2.48 and kurtosis 8.77 and 8.53, also presenting leptokurtic distribution with positive kurtosis and negative skewness. Sample 11 showed the highest blue mean value of 158, 48.6 standard deviation value, with skewness 0.86 and kurtosis 2.60. This may be interpreted as negative skewness but the kurtosis is platykurtic. In contrast, sample 1 demonstrated the lowest blue value of 140.2 with standard deviation of 78.5 and 1.97 skewness and 6.05 kurtosis with leptokurtic distribution. The above statistical analysis indicates that for sample 1 (Fig. 5), modification in pigmentation distribution remains the lowest compared to the other samples inoculated with the Xanthoria parietina species which may be attributed to the living cells retaining their nutrients and functional exchanges between the fungal and photosynthetic components. Sample 2 contains cellulose fibre scarva, methyl cellouse and chitosan, same particles used in sample 1. In addition to the above particles, sample 1 substrate also contains glycerine and additive. It may therefore be possible that the glycerine and additive contributed to the living cells presenting lowest pigmentation modifications and thus retaining their original living state and metabolic activity.

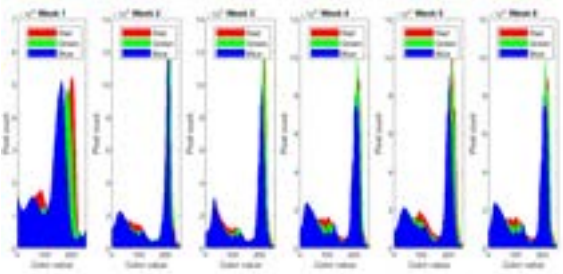

Figure 5: Plots depicting pigmentation distribution changes over 6 consecutive weeks for sample 1.

A similar trend can be observed in regard to the species Parmotrema perlatum. Sample 14 presented the highest red mean value of 169.6, standard deviation of 58.3, a negative skewness of 1.65 and a positive kurtosis of 4.46 with leptokurtic distribution. Sample 8 showed the highest green mean value of 165.4, standard deviation of 45.1, a negative skewness of 1.69 and a positive kurtosis of 5.80 with leptokurtic distribution. Finally, sample 12 showed the highest blue mean value of 157.2, standard

deviation of 45.8, a negative skewness of 0.86 and a kurtosis of 2.22 with platykurtic distribution. On the contrary, sample 9 displayed the lowest red, green and blue mean values of 155.2, 148.7 and 139.9 (Fig. 6). The standard deviation values were 73, 78.3 and 79 respectively, with negative skewness of 2.84, 2.62 and 2.44 and positive kurtosis of 10.67, 9.45 and 8.92, also with leptokurtic distribution. From the above analysis, it is demonstrated that from the substrates inoculated with Parmotrema perlatum lichen species, sample 9 displayed the lowest pigmentation distribution modifications and therefore its substrate might have contributed to the living lichens maintaining their original state and metabolic activity in comparison to the other samples. By comparison of the substances used in the samples, it may be possible that this behaviour is attributed to Bio bean and Additive and/or Fine Hemp Shive.

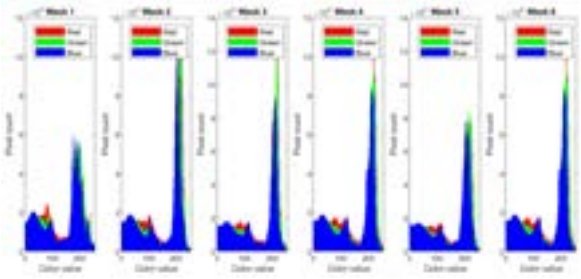

Figure 6: Plots depicting pigmentation distribution changes over 6 consecutive weeks for sample 9.

Lastly, for the species Flovoparmelia caperata, sample 14a showed the highest red mean value of 173, standard deviation of 46.7, a negative skewness of 1.08 and a negative kurtosis of 2.73 with platykurtic distribution. These findings are consistent with sample 14 discussed above, where the same substrate is inoculated with the lichen species Parmotrema perlatum and also show the highest red mean value. It is therefore possible that the particles used in substrates 14 and 14a have a maximum impact on the lichen species and more specifically on their metabolic activity. Table 1 shows in detail the substances used for each sample. Sample 14a also presents the highest green mean value which is 167.1 with 49.2 standard deviation, a negative skewness of 0.70 and a negative kurtosis of 2.16 with platykurtic distribution. Finally, three samples showed the same blue mean value of 156.2 which was the highest between the discussed samples of this lichen type. These samples are 3, 13 and 14. Standard deviation is 59.3, 45.1 and 53 respectively. The skewness is negative for all three samples and is 1.58, 1.43 and 0.45 respectively. The kurtosis is positive for samples 3 and 13 and is 4.28, 3.80 with a leptokurtic distribution and negative for sample 14 with a value of 2.34 and platykurtic distribution. It is therefore evident that the substrate components of sample 14 may impact most the metabolic activity of the lichens and therefore exhibit highest change of the pigmentation distribution. Regarding the lowest mean values, sample 5 demonstrated the lowest mean values for all three pigments (Fig. 7). These are 154.5, 148.9 and 141, showing standard deviation of 74.1, 79.8 and 80.9 respectively. The skewness is negative and the values are 2.99, 2.87 and 2.50 for red, green and blue pigments. The kurtosis is positive and is 12.20, 11.39 and 9.11 with a leptokurtic distribution.

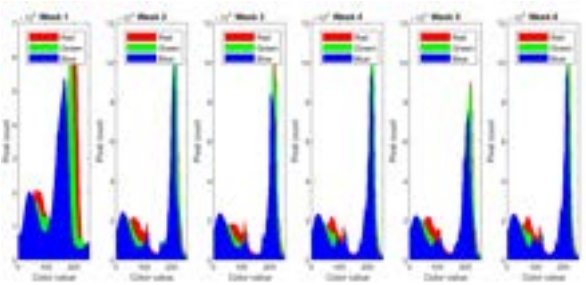

Figure 7: Plots depicting pigmentation distribution changes over 6 consecutive weeks for sample 5.

4. DISCUSSION

Our findings demonstrated that three combinations of lichen species-substrates presented optimal colonisation (1, 2, 11) and were therefore selected as most suitable for 3D printing using the system mentioned above (Fig. 8). Additionally, the data analysis contributed to a better understanding of the substrate-lichen correlation and more specifically the metabolic activity change over time. We can report that the three samples that showed lowest change of the pigmentation distribution are 1, 5, 9 and therefore their metabolic activity was more similar to their initial state than the other samples. On the other hand, most pigmentation distribution changes were observed in samples 2, 11, 14, 14a, 3 and 13 indicating that the metabolic activity of the living cells has been most impacted. We can therefore report that from the data analysis and physiological monitoring, sample 1 presents the most ideal candidate for the development of the new hybrid living material since it exhibits excellent colonisation behaviour and less changes in its metabolic activity over time. Further research needs to be undertaken to better understand the impact of the concentrations of the substrate components on the metabolic activity, the implication of metabolic changes to the living cells and substrate as well as the specifics of the substances used so that a more accurate mapping can be achieved.

5. CONCLUSION

This research project marks a ground-breaking investigation of incorporating lichens into substrate matrices aimed at robotic 3D printing. To our best

knowledge, this is the first time that such approach has been tested, opening up exciting opportunities for the development of a new class of hybrid materials using living components that are truly resilient to environmental stress, sustainable, can self- assemble, self-heal and self-regenerate. Our findings so far contribute to a better understanding of lichen species- substrates correlations as well as 3D printing approaches that can lead to the production of lichen hybrid living materials with scalability potentials for applications in the building industry through integration and self- assembly of their components.

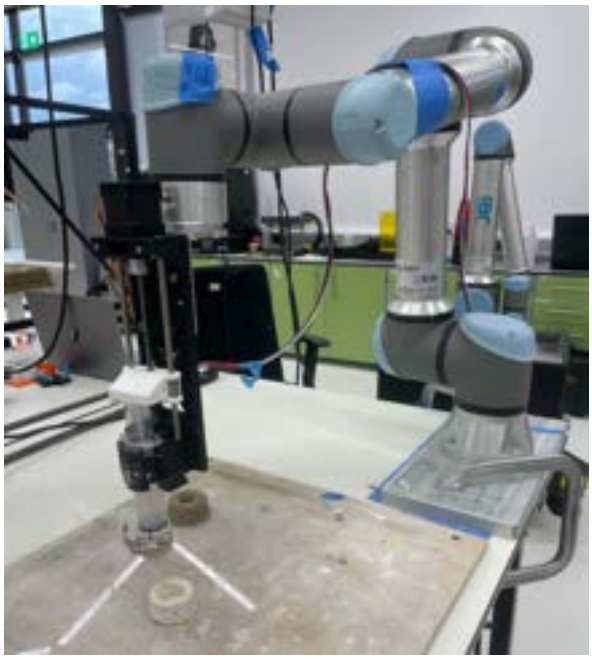

Figure 8: Small scale 3D printing studies of optimal substrates for lichen inoculation.

Following the above studies, we will use our findings for the next phase of this research project which includes the assessment of the responsiveness of the developed hybrid living materials to air pollutants exposure. For assessing gas absorption, the inoculated 3D printed hybrid living materials will be subjected to distinct gases in bespoke chambers and quantitative data will be collected for analysis of absorption. The findings of this phase will be included in the conference presentation. Challenges that need to be considered in this next research phase is minimising contamination risks and deterioration of the substrate material as well as monitoring the lichen hybrid materials for any pathogen growth.

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