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Photosynthetic textile biocomposites: Using laboratory testing and digital fabrication to develop flexible living building materials

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Abstract: Urban development and the construction industry account for a considerable proportion of global carbon dioxide (CO₂) emissions. Emerging biological materials, such as those proposed in this paper, seek to utilize the metabolic functions of living microorganisms to reduce some of the negative impacts of humans on the environment. The material explorations demonstrated in this paper propose a living photosynthetic carbon capture textile for the built environment. We demonstrate making practices that integrate living microorganisms within experimental methods of digital fabrication; specifically, harnessing photosynthetic microalgae that feed on waste and are capable of sequestering CO₂ from internal building settings. These new biocomposites incorporate flexible textile substrates, i.e. cotton, hessian, polyester, and canvas, which provide a range of algae laden matrices that continue to develop and change during the useful part of the material's lifecycle. This paper explores biological 3D printing fabrication processes and studies the development of mixtures that are compatible with the fabrication method and support microalgae (*Chlorella vulgaris*) metabolic processes. A range of incubation methods are assessed, highlighting the need for a support environment. The biocomposites' performance

is tested using imaging pulse amplitude modulation fluorometry (Imaging-PAM) to investigate changes in microalgae chlorophyll fluorescence over a 14 day period.

Keywords: flexible biocomposites, living materials, additive manufacture, bio-fabrication, photosynthetic textiles

1 Introduction

Modern construction practices and large-scale urban development have significantly impacted the ecology of the planet, from pollution and resource depletion to global warming and decreased biodiversity. Urban areas account for 70% of energy consumption worldwide, with buildings being responsible for the majority of carbon emissions [1]. It is in this context that designers and scientists look to nature to provide alternatives and models for symbiotic networks. Within bio-design, solutions are aimed at harnessing, and ideally enhancing, the natural metabolic functions of living organisms to produce sustainable end-products or to provide tangible ecosystem services. In the examples demonstrated in this paper, single cell photosynthetic microalgae are used as they utilize CO₂ and produce oxygen as a by-product. As living organisms, they come with their own set of requirements, including the need for light, suitable environmental pH and temperatures, and access to nutrients [2]. The work demonstrates how these conditions can be met so that built environments will benefit from their integration.

These new 'living materials' require a rethinking of building as well as fabrication practices, for example, printing using bio-gels has come to the forefront of materials research with applications within the pharmaceuticals and food industries driving research into the integration of living cells within digital fabrication processes [3,4]. Such applications typically focus on restoring human tissue or growing living cells *in vitro* and manufacturing food substitutes such as tissue-cultured meat for human consumption [5]. Within the field of bio-design, 3D printing with

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microorganism laden mixtures has become an area of exploration which includes the use of living mycelium for the manufacture of furniture [6], 3D printing bacterial cellulose as a building component [7], various algae-based projects that include experimental ink jet printing techniques [8], and algae-based 3D filaments for product manufacture [9], including self-healing properties [10]. This paper builds upon existing studies on algae ink development and 3D printing techniques for gel extrusion as part of digital fabrication [11–13], aiming to demonstrate how the developed bio-matrices can be used in conjunction with 3D printing. Methods of cultivating algae in gels form part of alternative practices for growing microalgae within low moisture content environments. Low moisture methods of cultivating microorganisms have previously been used in applications such as microbial fuel cells [14,15] and microalgae and cyanobacteria biocomposites using loofah [16] and ceramic [17] substrates. These approaches enable a greater density of cells to be grown within a smaller area, thereby significantly reducing the need for water and space [18].

Potential architectural applications of flexible photo-synthetic materials include internal environment finishes such as screens, wallpaper, and signage. Office environments and large public buildings provide appropriate conditions for such organisms to thrive by offering constant temperatures, a regular light cycle with high light intensity, and large surface areas. The eukaryotic green (Chlorophyta) microalga, *Chlorella vulgaris* (*C. vulgaris*), was used due to its compatibility with such environments and its resilience compared to many other microalgae and cyanobacteria. Microalgae are preferred organisms for CO₂ fixation in such environments as they capture inorganic carbon from low atmospheric CO₂ concentrations [19] and grow in a variety of settings difficult to populate by terrestrial plants [20]. In nature, such low moisture growth environments are referred to as biofilms [21] that contain a range of substances including living cells, secreted polymers, absorbed nutrients, metabolites, cell lysis products, and particles from the environment [22].

However, many biofilms can be short lived and are not mechanically robust [23]. In this paper, low moisture matrices (biocomposites) are developed that go beyond natural biofilms by integrating biocoatings with textiles. The flexible nature of the materials (cotton, hessian, polyester, and canvas) enables a wide range of applications that can be adapted by optimizing the morphology of the individual component in a lightweight system that acts as a breathing skin. The textiles were chosen based on considerations regarding sustainability, availability, and potential future design application. The textiles vary in texture, absorbency, density, and strength. This paper seeks to establish if the textile's properties impact the chlorophyll fluorescence of the microalgae (used as a proxy for algae health) over a period of two weeks, providing an adequate timeframe to infer if the substrate and matrix combinations can support cell viability and growth.

2 Method

The methods utilized in this paper are based on existing protocols used to develop algae-based biocomposites [16]. The study comprises two parts; the first seeks to establish the compatibility between four textile types (cotton, hessian, polyester, and canvas) and a range of matrices that contain living algae; the second part outlines the 3D printing process and matrix adjustment for extrusion compatibility. The bio-gel matrices incorporate the use of kappa-carrageenan (a hydrocolloid polymer extracted from red seaweeds), chitosan (a polymer commonly extracted from the exoskeletons of marine crustaceans), Aloe vera (extracted from the succulent plant *Aloe barbadensis*), and a clay-based paint binder (Auro 331) (ingredient information provided in Table 1). The study was split into three sequential stages; (1) closed testing of textile compatibility, (2) open testing of textile compatibility, and (3) 3D printing with bio-gels. Each stage of testing informed the next set of experiments.

Table 1: List of primary bio-gel components and their composition

Ingredient	Product information
Kappa-carrageenan Auro clay paint	Linear sulfated polysaccharides extracted from edible red seaweeds [24] Water, clay, mineral fillers, Replebin®, titanium dioxide, cellulose, surfactants made from rapeseed oil and castor oil, potassium, silicate, silicates, mineral pigments [25]
Aloe vera gel	Aloe barbadensis leaf juice, aqua, Cyamopsis tetragonoloba (guar) gum, xanthan gum, glycerin, sodium levulinate, sodium anisate, sodium phytate [26]
Chitosan	A linear polysaccharide composed of randomly distributed β-linked D-glucosamine and N-acetyl-D-glucosamine made by treating chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide [27]

2.1 Microalgae and bio–gel matrix preparation

The inoculating liquid algae culture was grown in full strength Blue–Green medium (BG11) comprising 1.5 g/L NaNO₃, 0.036 g/L CaCl₂·2H₂O, 0.075 g/L MgSO₄·7H₂O, 0.04 g/L K₂HPO₄, and 0.02 g/L Na₂CO₃ [28], at 18 ± 2°C with a 16:8 h light to dark photoperiod, at a light intensity of 2,500 lux (≈30.5 μmol m⁻² s⁻¹; [29]) provided by 30 W daylight-type fluorescent tubes (Sylvania Luxline Plus, n = 6). The cultures were placed in 50 mL Falcon tubes and centrifuged at 1,620 RCF (relative centrifugal force) for 10 min to produce a dense algae slurry. The algae slurry was mixed with the tested gel matrix at a ratio of 0.05 mL algae slurry per 1 mL of gel.

Each bio–gel matrix was prepared in 20 mL batches in 50 mL beakers. For the baseline kappa–carrageenan treatment (Table 2), 0.8 g of kappa–carrageenan (from >99.9% pure powder; Sigma Aldrich, UK) was added to 20 mL (0.04 g/mL) of a dilution series of BG11 (full strength, 75, 50, and 25% dilution) and stirred at room temperature. Once a gel-like consistency was achieved, 0.4 mL of *C. vulgaris* slurry was added and stirred until homogeneous. The quantity of kappa–carrageenan was reduced for later experiments which tested the performance of lower strength kappa–carrageenan gels. Aloe vera and Auro 331 Clay Paint binder (Auro Paint Company, UK) were tested as additives to the kappa–carrageenan baseline (Table 2). Ten grams of Aloe vera was added to the mixture. For the Auro paint, 0.64 g of kappa–carrageenan was dissolved in 16 mL of BG11 (0.04 g/mL) across the previous dilution series, to which 4 mL of Auro 331 was added (equivalent to 20% w/w Auro content). This process was repeated for all Auro paint mixtures, changing the quantities of kappa–carrageenan and Auro paint to produce the desired ratios and consistency. Chitosan treatments were prepared by dissolving food grade chitosan powder in a dilution series of BG11 following the addition of acetic acid (Table 2). The solution was stirred until an even viscous consistency was achieved prior to adding the algae slurry.

2.2 Textile substrate characterization

The absorbency of the textiles was measured (n = 3) by weighing dry 1 cm² samples of each textile that had been immersed in 3 mL of deionised water (dH₂O) for 1 h. Each textile sample (1 cm²) was placed on a glass slide with transparent tape to prevent the sample from slipping and imaged using a Leica DMI 8 microscope with LasX software, and thread size was measured. The pH of the textiles was determined using a standard water extraction method [30] by boiling 0.4 g of finely cut pieces (n = 3) in 75 mL of dH₂O for 10 min. After cooling to room temperature, the samples were drained and the pH of the water was tested using a pH meter (Mettler Toledo Seven Compact) relative to a control of dH₂O that had undergone the same process.

2.3 Closed lid testing: textile and bio–gel compatibility

The purpose of this assay was to develop a viscous matrix that was compatible with *C. vulgaris* and to assess the performance of this bio–gel once applied to the different textiles, forming biocomposites. The experimental treatments are outlined in Table 2. Textile samples (1 cm²) were placed in 24-well plates with 0.2 mL of BG11 (100, 75, 50, and 25% strength). The following treatments were run: (1) textile-gels containing algae, i.e. the biocomposite, (2) textile-gels without algae, i.e. non-biological controls, (3) algae suspension controls, (4) gels with algae, and (5) gels only. Each treatment was in triplicate (Figure 1). The gel coatings were deposited using a spatula, by applying 0.3 g of gel per sample. The well plates were sealed with a transparent lid to minimize any effect of evaporation or biological contamination and incubated at 18 ± 2°C with a 16:8 h light:dark cycle with 2,500 lux. Cell viability was assessed using an imaging pulse amplitude-modulated

Table 2: Bio–gel mixtures within a closed setup and method of incubation

Bio–gel mixture	Method of incubation	BG11 nutrient dilution (%)	Textiles
Kappa–carrageenan 0.4 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Closed lid, 24-well plate, 1 cm ² textile samples	100, 75, 50, 25	Cotton, polyester, hessian, canvas
Kappa–carrageenan 0.4 g/10 mL, 80% w/w BG11, 20% w/w Auro Clay Paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Closed lid, 24-well plate, 1 cm ² textile samples	100, 75, 50, 25	Cotton, polyester, hessian, canvas
Kappa–carrageenan 0.4 g/10 mL, 50% w/w BG11, 50% w/w Aloe vera gel, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Closed lid, 24-well plate, 1 cm ² textile samples	100, 75, 50, 25	Cotton, polyester, hessian, canvas
Chitosan 0.6 g/10 mL, acetic acid 0.3 mL/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Closed lid, 24-well plate, 1 cm ² textile samples	100, 75, 50, 25	Cotton, polyester, hessian, canvas

Figure 1: (Left) Closed lid incubations in 24-well plates with four BG11 nutrient dilutions, (right) open lid incubations in 6-well plates with full strength BG11.

fluorometer (Imaging-PAM M-Series; Walz GmbH) which quantifies the level of chlorophyll fluorescence using intense light pulses to stimulate photosystem II [31]. Data were collected every 2 days for 14 days by opening each well plate and applying 1 μ s pulses of 660 nm (pulsed LED, 0.5 μ mol quanta m⁻² s⁻¹) to incite photosynthesis using 16 red LEDs (660 nm) and 16 NIR LEDs (780 nm) [32].

2.4 Open testing: textile and bio-gel compatibility in an uncontrolled environment

This stage investigated the behaviour of the biocomposites during an open lid incubation, as well as their response

to stresses such as prolonged drying. The same treatments were run as per the closed testing trial. The gel coatings (Table 3) were deposited using a spatula, by applying 0.8 g of gel per sample. Textile samples (2 cm², $n = 3$) were placed in 6-well-plates with 1 mL of BG11. The well plates were left open and incubated under the same conditions as the closed test (14 days and assessed using imaging-PAM). The samples were rehydrated with dH₂O using a spray bottle delivering 1 mL per sample every 24 h. Separately, a combination of kappa-carrageenan and Auro Clay Paint biocomposites was further tested for performance over a range of drying times (24, 48, 72, and 96 h; Table 3). The drying periods enabled the point whereby drying may begin to detrimentally affect cell health and thus biocomposite performance.

Table 3: Bio-gel mixtures within open setup and method of incubation

Bio-gel mixture	Method of incubation
Kappa-carrageenan 0.4 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Open lid, 6-well plate, 2 cm ² textile samples
Kappa-carrageenan 0.4 g/10 mL, 80% w/w BG11, 20% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Open lid, 6-well plate, 2 cm ² textile samples
Kappa-carrageenan 0.4 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Dried for 24 h prior to rehydration with 3 mL BG11, open lid, 6-well plate, 2 cm ² textile samples
Kappa-carrageenan 0.4 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Dried for 48 h prior to rehydration with 3 mL BG11, open lid, 6-well plate, 2 cm ² textile samples
Kappa-carrageenan 0.4 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Dried for 72 h prior to rehydration with 3 mL BG11, open lid, 6-well plate, 2 cm ² textile samples
Kappa-carrageenan 0.4 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Dried for 96 h prior to rehydration with 3 mL BG11, open lid, 6-well plate, 2 cm ² textile samples
Chitosan 0.7 g/10 mL, acetic acid 0.2 mL/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	Open lid, 6-well plate, 2 cm ² textile samples

Figure 2: (Left) 3D printed algae cells in a kappa-carrageenan mixture on hessian during closed lid incubation, (right) 3D printing with 0.6 mm nozzle on wet cotton using living algae in Auro Clay Paint.

2.5 Gel development for 3D printing and extrusion tests

During this stage of testing, two mixtures that had successfully formed a paste-like consistency and did not present issues of biological contamination were further developed to match the requirements of the 3D printing equipment to enable controlled extrusion. The 3D printer was an air pressure-based extrusion printer (Lutum 4.5; VormVrij) as shown in Figure 2. The pressure-based system required a minimum pressure of 10 psi to be maintained, which required a lower viscosity than the previously tested consistency. This presented a challenge of decreasing the viscosity of the mixtures, while maintaining a favourable growth environment for the algae. This part of the study compared fabrication and subsequent incubation of two different gels including a kappa-carrageenan and full strength BG11 mixture as well as the Auro 331 Clay Paint mixed with BG11 and kappa-carrageenan, both of which remained wet for the duration of incubation (Table 4). Three bio-gel patterns were printed onto 30 cm × 10 cm samples of each of the four textiles (cotton, hessian, polyester, and canvas). The process was

repeated with control samples without living cells. Samples were incubated in open 90 mm Petri dishes under the same incubation conditions as the compatibility studies. Following 3D printing with the Auro binder and kappa-carrageenan mixtures, the samples were dried at 20°C for 24 h prior to rehydrating with 5 mL of dH₂O via spraying. Noticeable shrinkage occurred during the drying process, resulting in flaking and peeling from the textile substrate. The experiment was repeated without drying the mixture post-printing and the textile substrates were immediately hydrated with 5 mL of dH₂O after printing and incubated with a closed lid in 90 mm diameter Petri dishes. The samples were sprayed with 3 mL of dH₂O every other day to reduce evaporation. In the case of the kappa-carrageenan mixture, the samples were sprayed with 1 M potassium chloride solution immediately after printing to help stabilize the gel and to prevent distortion.

2.6 Statistical analysis

The Anderson–Darling test was used to test whether the data were normally distributed [33]. All data were non-

Table 4: 3D printing mixtures tested, extrusion conditions, and matrix behaviour during mechanical extrusion

Bio-gel mixture	Extrusion pressure (psi)
Kappa-carrageenan 0.6 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10
Kappa-carrageenan 0.8 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10
Kappa-carrageenan 1 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	20
Kappa-carrageenan 0.6 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10
Kappa-carrageenan 0.8 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10
Kappa-carrageenan 1 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	20

normally distributed and therefore two tests were applied for single and two factor datasets. The non-parametric Scheirer–Ray–Hare test was used for datasets with two factors – this is equivalent to two-way Analysis of Variance. The test assesses variance between two factors, which determines whether or not the interaction between the two has a bearing, with P -value of ≤ 0.05 indicating that the interaction between the two is not significant and therefore does not affect the outcome [34]. The non-parametric Kruskal–Wallis test was applied to datasets with a single factor, which assesses the statistical significance of three or more independent sets by comparing the median values [35].

Anderson–Darling tests and Kruskal–Wallis tests were conducted using Minitab 18, while Scheirer–Ray–Hare tests were performed using RealStatistics add-in for Microsoft Excel.

3 Results

3.1 Material characterization

With the exception of canvas, a smaller thread size resulted in lower absorbency (Table 5). There was no relationship between pH and thread size or absorbency.

3.2 Closed testing: textile and bio-gel compatibility

Most of the biocomposites supported either a gradual increase in chlorophyll fluorescence with time or enabled *C. vulgaris* to maintain the initial level of chlorophyll

Table 5: List of textiles and material properties

Textile type	Microscopic images of textile thread ($\times 5$ magnification)	Thread diameter (μm)	pH	Absorption ($\text{mL}/1\text{ cm}^2$)
Cotton		213 (mean StDev = 1.960)	7.66 (mean StDev = 0.213)	0.0429 (mean StDev = 0.0021)
Polyester		322 (mean StDev = 22.360)	8.36 (mean StDev = 0.247)	0.1269 (mean StDev = 0.0080)
Canvas (300 gsm)		367 (mean StDev = 43.848)	7.40 (mean StDev = 0.139)	0.0930 (mean StDev = 0.0076)
Hessian		1,115 (mean StDev = 157.849)	7.32 (mean StDev = 0.062)	0.1680 (mean StDev = 0.0138)

Figure 3: Closed incubation cotton samples with the following matrices: Aloe vera (mean StDev = 0.049) and control (mean StDev = 0.038), chitosan (mean StDev = 0.004) and control (mean StDev = 0.002), binder (mean StDev = 0.027) and control (mean StDev = 0.045), carrageenan (mean StDev = 0.014) and control (mean StDev = 0.022) in full strength BG11 media.

fluorescence (Figures 3 and 4). The exception was the chitosan containing biocomposite, which experienced a rapid decline in fluorescence by day 2; this was matched by the chitosan control indicating that the inclusion of acetic acid created an acidic pH that was incompatible with the algae (Figure 3). Textile type was a significant factor for those biocomposites that did support the algae for the duration of the trial (Scheirer–Ray–Hare, $n = 160$, d.f. = 4, $H = 20.82$, $P \leq 0.001$), whereas nutrient dilution was not significant (Scheirer–Ray–Hare, $n = 160$, d.f. = 3, $H = 5.91$, $P = 0.116$) and there was no significant interaction between the two (Scheirer–Ray–Hare, $n = 160$, d.f. = 4, $H = 0.66$, $P = 1.000$). Although biocomposites with the Aloe vera additive did support the algae over the duration of the experiment, these biocomposites experienced severe bacterial contamination that inhibited algae growth in the affected areas. Despite this, textile type was a significant factor (Scheirer–Ray–Hare, $n = 160$, d.f. = 4, $H = 131.50$, $P \leq 0.001$), unlike nutrient dilution (Scheirer–Ray–Hare, $n = 160$, d.f. = 3, $H = 0.369$, $P = 0.947$) and there was no interaction between the factors (Scheirer–Ray–Hare, $n = 160$, d.f. = 12, $H = 3.082$, $P = 0.995$).

The most promising biocomposites were kappa-carrageenan and full strength BG11 with the Auro Clay Paint additive. These biocomposites exhibited increased fluorescence in combination with cotton and polyester (Figure 5), with textile type a significant factor (Scheirer–Ray–Hare, $n = 160$, d.f. = 4, $H = 16.77$, $P \leq 0.005$), whereas

Figure 4: Closed incubation of 20% w/w binder on textiles: cotton (mean StDev = 0.032), canvas (mean StDev = 0.030), polyester (mean StDev = 0.047), hessian (mean StDev = 0.047), control (mean StDev = 0.057), and carrageenan samples: cotton (mean StDev = 0.014), canvas (mean StDev = 0.029), polyester (mean StDev = 0.026), hessian (mean StDev = 0.028), control (mean StDev = 0.025) in full strength BG11 media.

nutrient dilution (Figure 5) was not (Scheirer–Ray–Hare, $n = 160$, d.f. = 3, $H = 3.94$, $P = 0.267$); there was a significant interaction between textile type and nutrient dilution (Scheirer–Ray–Hare, $n = 160$, d.f. = 12, $H = 25.65$, $P \leq 0.05$). A similar outcome was attained with the kappa-carrageenan biocomposites, with textiles type significantly affecting chlorophyll fluorescence (Scheirer–Ray–Hare, $n = 160$, d.f. = 4, $H = 90.25$, $P \leq 0.001$), but not nutrient levels (Scheirer–Ray–Hare, $n = 160$, d.f. = 3, $H = 0.57$, $P = 0.901$); however, in this case any interaction was not significant (Scheirer–Ray–Hare, $n = 160$, d.f. = 12, $H = 3.17$, $P = 0.994$).

3.3 Open samples testing: uncontrolled environment

Textile type was a significant factor for the 20% w/w Auro paint bio-gels incubated in the open setup (Kruskal–Wallis, $n = 40$, d.f. = 4, $H = 14.51$, $P = 0.006$); it was also significant for the 50% w/w Auro bio-gels that had been dried over 24 h (Kruskal–Wallis, $n = 40$, d.f. = 3, $H = 12.00$, $P = 0.007$) (Figure 6). Biocomposites with a shorter drying time exhibited more consistent fluorescence levels that increased throughout the 14 days period, whereas samples rehydrated after a longer drying time exhibited a sharp decline of chlorophyll fluorescence in the initial days following

Figure 5: Closed incubation of 20% w/w binder on cotton with various nutrient media dilutions: 100% BG11 (mean StDev = 0.027), 75% BG11 (mean StDev = 0.036), 50% BG11 (mean StDev = 0.044), 25% BG11 (mean StDev = 0.020).

rehydration and a rapid recovery thereafter (Figure 7). Despite this, drying time was not statistically significant (Kruskal–Wallis, $n = 32$, d.f. = 3, $H = 0.70$, $P = 0.873$). In the chitosan treatment, the acetic acid content was reduced to make the environment less acidic; however, to achieve a

similar viscosity, the quantity of chitosan was increased. This resulted in some bacterial contamination. Textile type was not significant (Kruskal–Wallis, $n = 40$, d.f. = 4, $H = 3.17$, $P = 0.530$). The kappa–carrageenan and BG11 biocomposites were not detrimentally affected by open air incubation and substantial shrinkage or severe drying were not observed. In this case, textile type was statistically significant (Kruskal–Wallis, $n = 40$, d.f. = 4, $H = 14.51$, $P \leq 0.05$).

Figure 6: Open incubation of carrageenan cotton (mean StDev = 0.038) and control (mean StDev = 0.014), 20% binder cotton (mean StDev = 0.039) and control (mean StDev = 0.013), 50% binder cotton (mean StDev = 0.098) and control (mean StDev = 0.022), chitosan cotton (mean StDev = 0.012) and control (mean StDev = 0.006).

3.4 3D Printing with bio-gels

When extruding the kappa–carrageenan and paint mixtures, the decreased viscosity affected the ability to extrude a consistent layer even when using higher pressure. Air pockets and a separation of water content due to the pressure caused more noticeable distortion in the lower viscosity mixtures; therefore, the kappa–carrageenan content had to be increased while maintaining a consistency that allowed for smooth extrusion. A higher kappa–carrageenan content also resulted in crumbling and issues of adhesion to the substrate post-printing. The mixture variations and pressures used in each instance are presented in Table 6.

During the printing process, the texture of the textiles was of particular interest with smoother textiles such as

Figure 7: Open incubation of 50% w/w binder cotton samples following drying times of 24 h (mean StDev = 0.098), 48 h (mean StDev = 0.067), 72 h (mean StDev = 0.042), and 96 h (mean StDev = 0.045).

cotton and canvas presenting more favourable surfaces for printing, whereas the larger thread size of hessian produced better adhesion but caused issues during printing with the nozzle being prone to catching on the threads.

The 3D printed kappa-carrageenan samples continued to support cell metabolism during the 14 days testing period, with an increase in chlorophyll fluorescence recorded for cotton. Textile type was significant

Table 6: 3D printing mixtures tested, extrusion conditions, and matrix behaviour during mechanical extrusion

Bio-gel mixture	Extrusion pressure (psi)	Nozzle size (Diameter in mm)	Comments
Kappa-carrageenan 0.6 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10	0.6	Continuous flow from nozzle prior to printing, occurring due to pressurization of canister
Kappa-carrageenan 0.8 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10	0.6	Steady flow upon extrusion, gel maintaining its structure without crumbling or uncontrolled distortion
Kappa-carrageenan 1 g/10 mL, BG11, <i>C. vulgaris</i> slurry 0.2/10 mL gel	20	0.8	Inconsistent follow upon extrusion, crumbling of extruded filament, and poor adhesion to textile surface
Kappa-carrageenan 0.6 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10	0.6	Continuous flow from nozzle prior to printing, occurring due to pressurization of canister
Kappa-carrageenan 0.8 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	10	0.6	Mousse-like consistency, steady flow, good adhesion to textiles
Kappa-carrageenan 1 g/10 mL, 50% w/w BG11, 50% w/w Auro clay paint, <i>C. vulgaris</i> slurry 0.2 mL/10 mL gel	20	0.8	Inconsistent flow, crumbling of filament, and poor adhesion

(Kruskal–Wallis, $n = 32$, d.f. = 3, $H = 21.17$, $P \leq 0.001$) for the closed lid kappa-carrageenan samples. Canvas and polyester samples exhibited greater fluctuations, although all combinations still imply supported microalgae survival (Figure 8).

There was a slight decline in chlorophyll fluorescence of the Auro paint set in the initial days; however, this was followed by a gradual increase in chlorophyll fluorescence over the remaining 12 days (Figure 9). Textile type was significant (Kruskal–Wallis, $n = 32$, d.f. = 3, $H = 21.17$, $P \leq 0.001$). Visible changes were observed in the mixture colour, with more saturated green areas emerging. There was less cell migration (movement of living algae cells) from the mixture onto the empty substrate regions compared to the carrageenan samples (Figure 8), with the exception of the canvas samples that exhibited a greater level of cell migration and the resolution of the printed pattern under I-PAM was severely affected, although visible pattern distortion did not occur.

The kappa-carrageenan and BG11 combination had a more scattered distribution of cells when viewed in I-PAM; however, unlike the Auro samples, they did not initially go through a consistent period of decline, although sudden fluctuations were evident in the cotton and canvas samples. When comparing the two sets of samples, bio-gel type was significant (Scheirer–Ray–Hare, $n = 64$, d.f. = 1, $H = 22.60$, $P \leq 0.001$), whereas textile type was not (Scheirer–Ray–Hare, $n = 64$, d.f. = 3, $H = 7.26$, $P = 0.064$).

4 Discussion

This study aimed to develop and test a range of flexible, textile-based microalgae biocomposites that could be integrated into the internal fabric of the built environment. A fundamental driver for this work was to provide an environment that supported microbial life (in this instance, the microalga *Chlorella vulgaris*), which would subsequently become part of the metabolism of the building, i.e. by sequestering CO₂ whilst releasing O₂, and potentially remediating wastewater. Aside from the functional aspect, the studies also sought to address the aesthetics of deploying biocomposites using extrusion 3D printing. *Chlorella* was chosen for its ability to flourish in temperatures similar to those offered by interior building environments (19–24°C), for its resilience to desiccation, and its efficient photosynthetic rate (some *Chlorella* species can reach efficiencies of more than 20% compared to the typical 1% efficiency of terrestrial plants [36]).

This study utilized imaging PAM fluorometry as a means to non-destructively measure in situ chlorophyll fluorescence. While being particularly helpful in visualizing cell migration and pattern distortion as well as pattern of growth, it does not measure the amount of carbon captured; this can only be inferred based on the cell's chlorophyll fluorescence levels that indicate a greater amount of photosynthetic cells and therefore more agents for CO₂ sequestration. Further research is necessary, for instance, testing the biocomposites as part of an airtight CO₂

Figure 8: 3D printed patterns on cotton using kappa-carrageenan (top) and Auro Clay Paint (bottom), image showing cell chlorophyll fluorescence in I-PAM, red and yellow indicate low levels of fluorescence of living photosynthetic cells, green and blue indicate higher levels, and black indicates a lack of living photosynthetic cells. The dense green areas on day 14 indicate cell migration outside of the printed area.

absorption system to quantify the captured carbon. One of the drawbacks of PAM fluorometry is that it is not able to measure the chlorophyll fluorescence inside an opaque

matrix; therefore it is only capturing chlorophyll fluorescence on the surface of an opaque matrix or parts of a translucent matrix. A chlorophyll extraction technique can potentially be used at the end of the test to compare with the data obtained from the imaging PAM.

The first set of tests helped establish differences in biocomposite performance (as measured by in situ chlorophyll fluorescence) based on the type of textile substrate. The initial findings indicated that textile type had a significant bearing on the development of the living cells. The nutrient levels built into the bio-gels indicated that full strength BG11 produced the most favourable results; however, all other dilutions ably supported the algae development throughout the test period.

During the two weeks of closed incubation tests, *C. vulgaris* embedded in the Auro binder with cotton had the best overall performance followed by the carrageenan-based matrices; [16] also used Auro binders to produce cyanobacteria loofah-based biocomposites and reported increased CO₂ absorption rates compared to suspension controls. For carrageenan-based matrices, enhanced biological performances have been widely reported in many studies particularly in wastewater treatment [37]. The less favourable gel matrices such as chitosan-based mixtures

Figure 9: Chlorophyll fluorescence levels of the kappa-carrageenan on the following textiles: cotton (mean StDev = 0.134), canvas (mean StDev = 0.086), polyester (mean StDev = 0.144), hessian (mean StDev = 0.043), and Auro paint 3D printed samples on textiles: cotton (mean StDev = 0.056), canvas (mean StDev = 0.053), polyester (mean StDev = 0.037), hessian (mean StDev = 0.060) over a 14 day period.

need further study to overcome the low pH of acetic acid and promote biocompatibility [38]. Tıǧlı et al. provide an alternative where the chitosan-based scaffolds were formed using a glycerol phosphate disodium salt as an ionic cross-linker while adding NaOH to increase the pH up to 7.0 after dissolving the chitosan in acetic acid; such an environment may provide a more favourable pH for algae growth [39].

The second set of tests demonstrated the resilience of the biomaterials, suggesting that they can be deployed in interior settings where it would be exposed to the air. However, this presents a risk of contamination or cell damage if other agents are present such as aerosols, dust particles, or large numbers of other microorganisms. During the rehydration process, a level of flaking was observed due to shrinkage caused by the kappa-carrageenan content. The kappa-carrageenan was added to aid the creation of a paste-like consistency which would be suitable for 3D extrusion; however, an alternative application that accommodates distribution of a less viscous solution, such as painting with a brush or a roller, would negate the need for kappa-carrageenan in the mixture and would reduce flaking.

This paper builds upon early research into bio-ink rehydration and mimicry of natural biofilm growth, by engineering thin layers of nontoxic materials that are capable of supporting a high cell density [40,41]. Nontoxic binders, for example in latex [16,42], provide a more appropriate matrix for cell cultivation, as hydrogels (although easily formed) possess a larger pore structure that is prone to the physical release of cells. Hydrogels also present a less stable environment for long-term storage of dormant cells as outlined by Flickinger et al. [42].

The incubation studies also provided insight into the potential distribution and storage of a living material in a dormant state. The dry Auro samples that were rehydrated after a range of timeframes demonstrate the potential for prefabricating such elements off-site and transporting at a distance, aiding the distribution process. However, cultivation in an open interior environment is also prone to sudden changes in moisture levels where the substrate could dry out, causing cell stress and matrix flaking. This suggests the need for an automated irrigation system that could detect such changes and maintain a minimal moisture level at all times, which may raise issues of needing an energy source that may negate the benefits of biological CO₂ sequestration. An alternative may be to utilize a protective cover and to develop a human maintenance protocol that may place a burden upon inhabitants. Both options need to be explored further, with the current study indicating that evaporation is a limiting factor.

In the final set of experiments, the consistency of the bio-gels was adapted to suit the limitations of the extrusion 3D printer, with high viscosity mixtures resulting in uncontrolled expulsion of material prior to initiating the printing cycle and lower viscosity mixtures causing blockages and crumbling of the matrix with poor adhesion to the textile substrate. Although detailed structural analysis of the printed biocomposites was not undertaken in the study, prior analysis using latex binders [16] suggests that dry cracked binders can be a source of adhesion and cell retention failure. An aspect of the 3D printed process that needs to be assessed is the effect of the nozzle size on cell viability as a larger nozzle, as observed by Unagolla and Jayasuriya, requires less pressure and is likely to result in less cell damage during fabrication and therefore better cell viability following extrusion [38]. The 3D printing process has created challenges, and issues of speed, cost, and gel pattern preservation have been identified as limiting factors for large-scale mass production and implementation [43]. Further testing is necessary to assess if the matrix would permit 3D extrusion of a geometry at a height as studied by Wang et al. [44] and its effect on the quality of adhesion to the substrate. The integration of the textile substrates into design needs to be studied as the samples of this study were incubated flat, with vertical incubation taken into account of gravity or twisting and tensioning of the material presenting areas of interest for future investigation.

5 Conclusion

This paper has demonstrated that *C. vulgaris* can be cultivated in a minimal moisture environment in a range of matrices and on a variety of textiles that lend themselves to an array of potential applications within the building fabric. The aim of the study was to develop a new type of living material that can be fabricated using digital 3D printing methods and to study the subsequent development of such materials in terms of cell development and migration demonstrating live behaviour that is not traditionally associated with materials used within the construction industry. The results indicate that living textile materials would need some form of maintenance schedule to prevent sudden moisture evaporation which could result in cell death and flaking of the matrix. Further investigation is necessary to design an artificial support environment and to develop ways of overcoming challenges of matrix shrinkage and flaking as well as to develop viable design solutions for integration within the built environment.

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