

# Development of bio-inspired multi-functional polymeric-based fibers (BioFiber) for advanced delivery of bacterial-based self-healing agent in concrete

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**Abstract.** The goal of this research is to develop innovative damage-responsive bacterial-based self-healing fibers (hereafter called BioFiber) that can be incorporated into concrete to enable two functionalities simultaneously: (1) crack bridging functionality to control crack growth and (2) crack healing functionality when a crack occurs. The BioFiber is comprised of a load-bearing core fiber, a sheath of bacteria-laden hydrogel, and an outer impermeable strain-responsive shell coating. An instant soaking manufacturing process was used with multiple reservoirs containing bacteria-laden, hydrophilic prepolymer and crosslinking reagents to develop BioFiber. Sodium-alginate was used as a prepolymer to produce calcium-alginate hydrogel via ionic crosslinking on the core fiber. The dormant bacteria (spore) of *Lysinibacillus sphaericus* was incorporated in hydrogel as a self-healing agent. Then, an impermeable polymeric coating was applied to the hydrogel-coated core fibers. The impermeable strain-responsive shell coating material was manufactured using the polymer blend of polystyrene and polylactic acid. The high swelling capacity of calcium-alginate provides the water required for the microbially induced calcium carbonate precipitation (MICP) chemical pathway, i.e., ureolysis in this study. The strain-responsive impermeable coating provides adequate flexibility during concrete casting to protect the spores and alginate before cracking and sufficient stress-strain behavior to grant damage-responsiveness upon crack occurrence to activate MICP. To evaluate the behavior of developed BioFiber, the swelling capacity of the hydrogel, the impermeability of shell coating, the spore casting survivability, and MICP activities were investigated.

## 1 Introduction

The performance of materials is dominated by irreversible chemical/physical degradation processes, which leads to thermodynamic, chemical, and mechanical instability of materials [1, 2]. The degradation mechanism is an interplay of intrinsic and extrinsic conditions, such as microstructural and compositional changes, time-dependent deformation, aggressive substance attacks, and harsh environments [1]. Implementation of degradation mitigation strategies can be expensive, time-consuming, and unpractical [3]. Since self-healing capability provides lifetime extension and enhanced mechanical integrity to the materials, researchers have shown growing interests in the nature-inspired self-healing functionality to integrate the self-healing properties into synthetic materials [4, 5]. Self-healing features in materials can prolong their service-life, improve performance efficiency of the materials, and have desirable economic attributes [6]. Furthermore, self-healing materials offer long-term

performance reliability particularly in limited accessible areas [7].

Quasi-brittle cementitious composites are susceptible to micro-cracks formation due to thermal, mechanically, and chemically induced damages [5]. The formation of microcracks adversely affects the mechanical integrity and durability of cementitious materials [2]. The damages induced to the concrete structures and infrastructures, due to the crack formation and propagation, impose hefty expenses on the governments and private sectors. As self-healing techniques reveal potential advantages in the service-life extension and resilience of cracked/fractured materials, numerous researchers have shifted their focus from damage prevention methods to damage management strategies, mainly self-healing, in concrete structures and infrastructures [8-12]. The unfavorable and harsh concrete environments e.g., high pressure, temperature, alkaline condition, and unavailability of sufficient oxygen, adversely affect healing agents' activity/survivability [13]. Several carrier techniques have been used to protect healing agents during their incorporation in concrete [14, 15]. In these carrier techniques, the occurrence of a crack can rupture the

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protective carrier and results in the release of healing agents into the crack space. Self-healing mechanism begins after the release and exposure to the triggering materials (such as calcium, urea, and/or nutrients). Wang et al. investigated using hydrogel capsule as bio-healing agents [16]. They utilized tri-block copolymer of poly (ethylene oxide) and poly (propylene oxide) as a swollen hydrogel, protecting bacterial agents in concrete. Jiang et al. manufactured shell/core capsules system by adding a composite shell structure using melamine phenolic resin with modified sodium alginate to a core made of epoxy resin as healing agent [17].

Based on previous studies [9, 18-20], there are various aspects of self-healing, including crack growth control and damage-responsiveness, that are not thoroughly covered in the literature. In particular, there is a need to develop a damage-responsive self-healing strategy that can provide dual functionality of damage growth management as well as damage self-healing. In addition, there is limited knowledge in the literature investigating methods to improve the survivability and long-term performance of bio-inspired healing agents. Hence, the main objective of this study is to develop bacteria-laden multi-functional fibers (hereafter called BioFiber) to integrate the damage-responsive self-healing feature into cementitious materials. As a bio-healing agent, spores were infused into the biocompatible hydrogel. The spores are long-lasting dormant bacteria, having higher resistance rate to the harsh environmental conditions. The vegetative cells resulting from germinated spores reveal higher rates of urea hydrolysis, and higher calcium carbonate precipitation, compared to vegetative cells that did not originate from spores. The finding of this study will be critical to efforts to fabricate bacteria-laden structures with tuneable physical/mechanical properties not only for concrete applications, but for a wide range of applications, such as ceramic-based self-healing or tissue engineering.

## 2 Methodology

### 2.1 Materials

The materials used for BioFiber manufacturing were categorized as: (i) polymeric core-fiber, (ii) spore-laden hydrogel, and (iii) copolymeric shell coating. For the core-fiber, polyvinyl alcohol (PVA) fiber was used, and the material/geometrical properties shown in Table 1.

**Table 1.** Polymeric core-fiber properties reported by the manufacturer.

| Material                             | Polyvinyl alcohol |
|--------------------------------------|-------------------|
| Length (mm)                          | 30                |
| Diameter $\mu$ ( $\mu\text{m}$ )     | 611               |
| Diameter $\sigma$ ( $\mu\text{m}$ )  | 19                |
| Tensile Strength (MPa)               | 800               |
| Elastic Modulus (GPa)                | 23                |
| Specific Gravity                     | 1.3               |
| Melting Point ( $^{\circ}\text{C}$ ) | 225               |
| Morphology                           | Single Filament   |

To load bacterial spores on the PVA core-fiber, calcium-alginate was used as a hydrogel carrier. We used alginate as a carrier for bacterial spores due to its biocompatible, hydrophilic, and tuneable swelling capacity. The calcium-alginate was produced via ionic crosslinking using calcium acetate solution with molarity of 0.259, and 2, 4, 8 wt.% sodium-alginate as a prepolymer. The concentration of sodium-alginate was 2, 4, 8 wt.% to investigate changes in the hydrogel swelling capacity and hydrogel thickness on the core-fiber. In this study, the vegetative cells of *Lysinibacillus sphaericus* MB284 were converted to endospores through thermal shock as an endospore formation method. The germination of spore is triggered by the presence of carbon and nutrient source, through which the spores re-enter the vegetative growth and cell division. In this study, the spores were revived in a culture medium of yeast extract (20 g/L) and urea (20 g/L) were used. The biomineralization of calcium carbonate was governed by urea hydrolysis pathways to produce carbonate ions and the use of calcium acetate (20 g/L) as a source of calcium ions in the medium.

For the polymeric-based shell coating, we have tested various lacquers and strain-responsive polymer systems to satisfy three criteria. First, the shell coating must be impermeable to protect the spores/alginate layer against uncontrolled release of spores and swelling of hydrogel. Second, the coating must have sufficient flexibility to withstand concrete mixing, and enough brittleness to be fractured upon cracking to release the bio-agents. Third, the ratio of shell layer thickness to diameter of the core-fiber loaded with spore/hydrogel must be minimized to achieve desired bridging functionality. To satisfy all the mentioned criteria, the shell coating materials were narrowed down to a blend of polystyrene (PS) and polylactic acid (PLA) after comprehensive experimental examinations. PS is hydrophobic brittle polymer while polylactic acid (PLA) is a relatively flexible polymer. A PS:PLA mass ratio of 1:1 was found to provide desired permeability, flexibility, and brittleness for shell coating materials. Chloroform was used as a solvent with the copolymer weight to solvent volume (w/v) ratio of 12.

### 2.2 BioFiber Manufacturing

To manufacture BioFibers, the first stage is to apply spore-laden alginate coating on the core-fibers. To carry out this stage, the core-fibers were first soaked in the sodium-alginate solution containing spores. Then, the core-fibers were soaked in high concentrated calcium acetate solution to crosslink the sodium-alginate. The soaking in both stages was instant immersion in the solution. After the spore-laden hydrogel was loaded on the core-fibers, the fibers were set to dry under ambient condition (23  $^{\circ}\text{C}$ ) for 24 hours. The last stage was to instantly soak the fibers in the PS:PLA/Chloroform solution and dry them for 2 hours under ambient condition.

## 2.3 Experiments

The experimental program was designed to investigate the performance of the developed BioFibers as a delivery system based on three criteria: (i) hydrogel water up-take performance, (ii) shell coating protecting performance, (iii) MICP self-activation performance. To study the performance of hydrogel, the swelling capacity of the spore-laden hydrogel coated on the core-fiber was calculated via gravimetric analysis. The weight of the hydrogel coated fibers were measured at dry and wet state, and the swelling ratio (g/g) was calculated based on Eq. 1. The fibers were submerged in solution with pH of ~3, 7, and 13, for elapsed time of 5, 10, 15, 30 minutes. After exposure, the excess water removed from the surface of the fibers using soft tissue wiping cloth and immediately weighed. Further, the hydrogel thickness on the core-fiber was measured using an optical microscope.

$$S_r = (W_{wet} - W_{dry}) / W_{dry} \quad (1)$$

where  $S_r$  is the swelling ratio,  $W_{wet}$  and  $W_{dry}$  are the weight of the samples in swollen and dried state, respectively.

To evaluate the shell coating performance, a survivability test was designed based on the color change of the pH indicator blended in the hydrogel. The hypothesis was that if the shell coating is permeable, the hydrogel could not be protected by the shell, and hydrogel swells and due to presence of pH indicator, the color of the BioFibers changes as it is immersed in concrete synthesized pore solution. For the pH indicator, 0.25% w/v of phenolphthalein was added to the spore/sodium-alginate solution. Phenolphthalein color changes from clear in acidic/neutral solution to purple in exposed to alkaline solution. The BioFibers were exposed to synthesized pore solution (~pH of 13), as a simulation of the concrete alkali condition, to explore the survivability of the shell coating against ingress of solution. In addition, the shell coating thickness on the hydrogel-coated core-fiber was measured using an optical microscope.

To investigate the survivability of BioFibers during concrete casting, the BioFibers were mixed with water and quartz sand using a hand mixing (HM) and vacuum mixer (VM) for 2 minutes. The mix design was based on a typical mortar mixture with the exclusion of cement, as 1, 33.69, and 65.31% volume fraction of BioFiber, water, and sand, respectively. After mixing, the BioFibers were separated, exposed to synthesize pore solution, and observed under optical microscope to detect possible crack/color change.

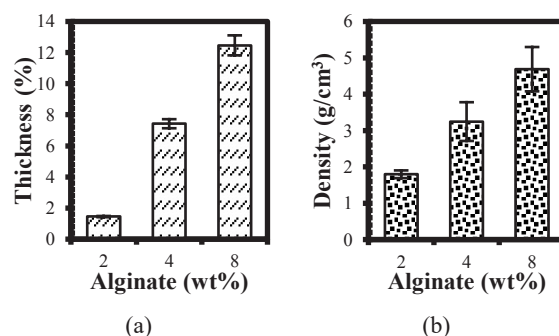
To understand the MICP activity, thermogravimetric analysis (TGA) was performed to quantify the amount of precipitated calcium carbonate in the BioFibers after 30 hours of exposure to nutrient and calcium sources. Both intact (as a reference) and cracked BioFibers were used. The precipitated solids were centrifuged, dried for 1 hour at 105 °C, and grinded for the TGA samples. A sample of 20-30 mg was taken from the solid powders and tested at 30-900 °C with the ramp rate of 10 °C/minute under nitrogen gas.

## 3 Results and Discussion

### 3.1 Spore-Laden Hydrogel Water Up-Take Performance

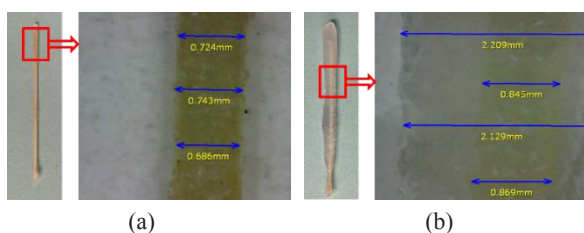
Sodium-alginate concentrations were varied at 2, 4, 8 wt.% to determine its effect on the hydrogel thickness, density, and swelling ratio. The hydrogel thickness percentage on the core-fibers are calculated based on the ratio of the thickness per average radius of the PVA core-fiber, and the data are shown in Fig. 1 (a). These results indicated that as the alginate concentration increased, a higher amount of hydrogel was loaded into the core-fibers. Since the viscosity of alginate increases with the increase of concentration [21], more sodium-alginate was able to be mounted on the surface of the PVA upon instant soaking, as shown in Fig. 1 (b) where an increase in specific gravity can be seen. The average thickness of hydrogel was increased from 1.46 to 12.46% for the alginate concentration of 2 to 8 wt.%.

The swelling characteristics of the spore-laden calcium-alginate hydrogel was determined by gravimetric analysis. An example of spore-laden hydrogel on the PVA core-fiber before and after swelling is shown in Fig. 2. The results of swelling ratio for 15-minute exposure to the neutral solution (pH of 7) are shown in Fig. 3 (a). As it is observed in this plot, higher sodium-alginate solution resulted in higher swelling ratio, which is mainly contributed to the higher amount of hydrogel loaded on the core-fibers. Higher concentration of sodium-alginate resulted in a solution with higher viscosity, which subsequently led to higher amounts of hydrogel being adhered to the core-fibers. To determine the effects of alginate concentration on swelling capacity of the hydrogel alone, the swelling ratio was re-calculated by subtracting the initial weight of the core-fibers, served as a normalization of the weight of hydrogel in swollen state to its dry condition. The swelling capacity results for the hydrogel itself are shown in Fig. 3 (b), indicating that the swelling ratio decreased as alginate concentration increased. This observation is due to achieving higher degree of crosslinking in alginate using higher concentration of sodium-alginate solution, which resulted in lower swelling ratio. It should be noted that the swelling of the core was found to relatively be negligible in comparison to the adhered hydrogel.

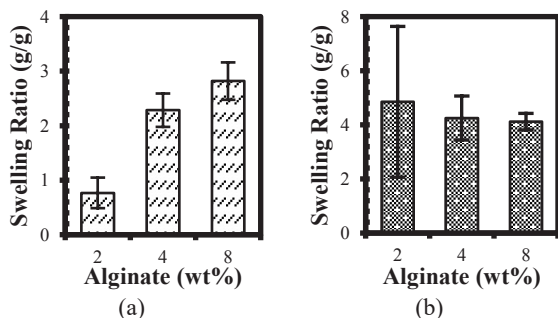


**Fig. 1.** (a) Hydrogel thickness, (b) density of the hydrogel coated core-fiber.

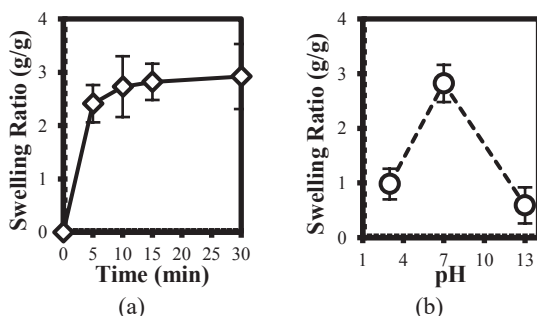
Furthermore, the effects of exposure time and pH of the solution on the swelling capacity of the BioFiber were investigated. Fig. 4 (a) shows the swelling ratio of the hydrogel made with 8 wt.% alginate over different exposure time to the solution with pH of 7. The swelling ratio increased over time and reached a plateau after 15 minutes exposure. In Fig. 4 (b), the effect of acidic, neutral, and basic solution on the swelling ratio of 8 wt.% alginate hydrogel was studied. The results showed a significant decrease as the pH shifted from neutral condition. In the acidic condition, the low swelling ratio can be attributed to the high repulsion of anion–anion COO<sup>-</sup> groups. In the basic conditions, i.e., synthesize pore solution, the presence of counter ions (Na<sup>+</sup> and K<sup>+</sup>) and non-ionic hydrophilic OH<sup>-</sup> can be the swelling loss in high pH solution [22].



**Fig. 2.** Spore-laden hydrogel (8 wt.% alginate) on PVA core-fiber, (a) before swelling, (b) after swelling.



**Fig. 3.** Swelling ratio: (a) core-fiber coated with spore-laden hydrogel, (b) spore-laden hydrogel (the weight of the PVA core-fiber is excluded in the calculation).



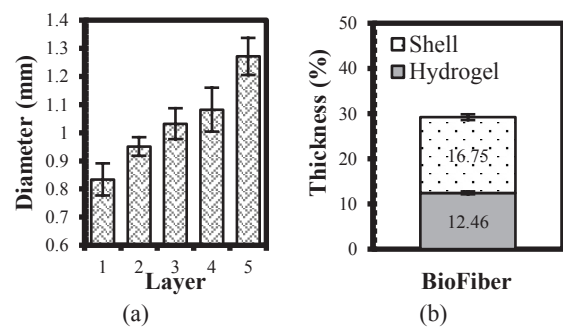
**Fig. 4.** Hydrogel made with 8 wt.% alginate; (a) swelling ratio vs exposure time at pH=7, (b) swelling ratio vs. solution pH.

### 3.2 Shell Coating Protecting Performance

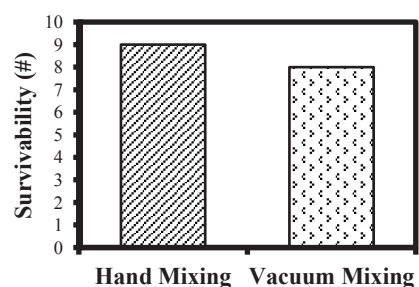
To develop Biofibers, it is critical to create an impermeable coating to protect spores and hydrogel before the occurrence of cracks in concrete. It is also critical that the coating is damage-responsive, where the occurrence of crack can break the coating material and expose hydrogel and spores to nutrients, water, and urea

for MIPC activation. The proposed copolymer system made of PS and PLA (1:1 mass) with 12 w/v copolymer/solvent ratio was found to be a promising blend for the shell coating based on comprehensive experimental work where its brittleness and ductility was tailored for damage-responsive functionality. In order to tune the shell coating thickness on the hydrogel/PVA fibers to survive the solution ingress, 1 to 5 coating layers were applied. Since previous results regarding hydrogel performance indicated higher swelling ratio of hydrogel crosslinked with 8 wt% sodium-alginate, the shell coatings were applied on the core-fibers coated using the 8 wt% alginate concentration. The effects of coating layers on the BioFiber final diameter are shown in Fig. 5 (a). The BioFiber diameter varied from 0.834 to 1.271 mm, as the number of the shell layers varied from 1 to 5, respectively. To calculate how much thickness each layer of shell coating added to the core-fiber, the average value for shell copolymer upon single layer application is shown in Fig. 5 (b). In this figure, the average thickness of the shell coating and the average hydrogel thickness were shown separately.

In addition to shell thickness measurements, it was important to identify the minimum number of shell layers required to pass the solution ingress survivability test. The results indicated that the minimum of about 48% shell thickness was required on the hydrogel/PVA fibers to meet the impermeability requirements, which is 3 layers of shell coating. The next criteria for the shell coating was to survive the casting process of the concrete. For this stage, a set of 10 BioFibers were used for the casting process. Fig. 6 (a) shows the number of BioFiber survived the casting process out of 10 tested samples while keeping their impermeability.



**Fig. 5.** (a) BioFiber diameter with hydrogel/shell coating, (b) average thickness of hydrogel and each layer of shell coating.



**Fig. 6.** Concrete casting survivability

### 3.3 MICP Self-Activation Performance

The final stage was to perform thermogravimetric analysis to quantify the amount of calcium carbonate precipitated in the BioFibers upon shell cracking and 30 hours of exposure to yeast extract (20 g/L), urea (20 g/L), and calcium acetate (20 g/L). For this experiment, an intact and cracked BioFiber made with 8 wt.% alginate, PS:PLA (1:1 mass%), copolymer/solvent of 12 w/v, and 3-layer shell coating were used. The thermogravimetric results for the intact and cracked BioFibers were illustrated as weight loss curve (TGA) and derivative curve (DTG) in Fig. 7 (a) and (b), respectively. As shown in the results, the weight loss in the temperature range of 30-105 °C was associated with remaining moisture in the samples. The weight loss between 200-600 °C is associated with the organic matter decomposition into residue solid and gases. The final weight loss in the temperature range of 600-800 °C is associated with the decomposition of calcium carbonate (CaCO<sub>3</sub>) to calcium oxide (CaO). Based on the following equations, the amount of calcium carbonate was calculated.

$$W_{CaCO_3} (g) = WL_{CaCO_3} \times (M_{CaCO_3} / M_{CO_2}) \quad (2)$$

$$NW_{CaCO_3} (\%) = W_{CaCO_3} / (W_{Initial} - W_{Moisture}) \quad (3)$$

where  $WL_{CaCO_3}$  is the weight loss between 600-800 °C,  $M_{CaCO_3}$  and  $M_{CO_2}$  is the molar weight of calcium carbonate and carbon dioxide. In order to remove the moisture in the results, the normalized values ( $NW_{CaCO_3}$ ) were calculated using the initial weight of the TGA sample ( $W_{Initial}$ ) minus the weight loss of moisture ( $W_{Moisture}$ ).

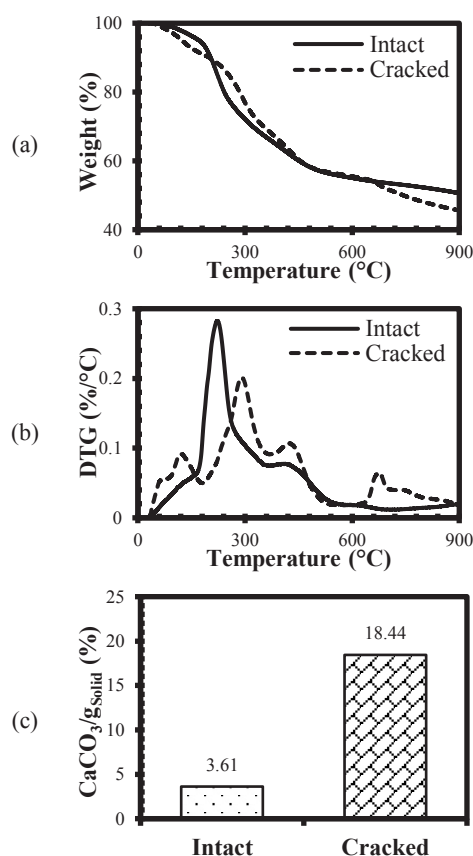
The results for intact and cracked BioFibers were shown in Fig. 7 (c). The amount of calcium carbonate was normalized to the amount of the solid powder that was tested for TGA minus moisture. The results indicated that the cracked BioFibers produced significant calcium carbonate compared to intact ones. Despite the intact BioFiber being expected to show zero calcium carbonate precipitation, a small amount was likely observed due to imperfections at the edges of the BioFibers or external source of contaminations on the shell coating.

### 4 Summary and Conclusion

This study investigates the development and performance of innovative multi-functional damage-responsive polymeric-based fibers, entitled BioFibers. The primary purpose of BioFiber is to act as a delivery system for the bacterial-based self-healing agent into cementitious materials. According to the results obtained in this study, following conclusions can be drawn:

- The polystyrene/polylactic acid copolymer shell coating proved to integrate the damage-responsive feature into the BioFiber. This copolymer coating performed as a protectant of spore release before crack occurrence. The thickness of the hydrogel and

shell coating was tailored to achieve the minimum value that meets the performance requirements, i.e., hydrogel high swelling and shell impermeability.



**Fig. 7.** Thermogravimetric results: (a) TGA, (b) DTG, (c) quantified amount of calcium carbonate precipitated.

- The BioFibers showed acceptable survivability during concrete casting process where more than 80% of BioFibers survived during either hand mixing or vacuum mixing.
- The cracked BioFibers showed successful release of spores as exposed to nutrient/calcium media, producing sufficient microbial induced calcite precipitation for crack healing.

While the current study showed promising results regarding the performance of the BioFibers, further studies are required to determine the behavior of this innovative multi-functional fibers in the concrete. In addition, understanding the healing mechanisms, the MICP kinetics, and the potential fracture properties enhancements can be the objectives of future studies.

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