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# X-ray computed tomography proof of bacterial-based self-healing in concrete

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ABSTRACT

Self-healing strategies are regarded as a promising solution to reduce the high maintenance and repair cost of concrete infrastructures. In the present work, a bacterial-based self-healing by use of hydrogel encapsulated bacterial spores (bio-hydrogels) was investigated. The crack closure behavior of the specimens with/without bio-hydrogels was studied quantitatively by light microscopy. To have a view of the self-healing inside the specimens, a high resolution X-ray computed microtomography (X-ray μCT) was used. The total amount and the distribution of the healing products in the whole matrix were investigated. This study indicates that the specimens incorporated with bio-hydrogels had distinct improved healing efficiency compared to the reference ones with pure hydrogel only. The healing ratios in the specimens with bio-hydrogels were in the range from 70% to 100% for the cracks smaller than 0.3 mm, which is more than 50% higher than for the ones with pure hydrogel; and the maximum crack bridging was about 0.5 mm (in 7 d), while pure hydrogels only allowed healing of cracks of about 0.18 mm. The total volume ratio of the healing product in the specimens with bio-hydrogels amounted to 2.2%, which was about 60% higher than for the ones with pure hydrogel (1.37%).



## 1. Introduction

Self-healing is a potential solution to obtain a sustainable concrete, since it would reduce the high maintenance and repair costs of concrete infrastructures [1]. Due to the limited autogenous healing capacity of concrete itself, extra healing agents are needed to enhance the self-healing properties. Among the currently investigated self-healing strategies, the microbial-based strategy for self-healing concrete cracks is an emerging field. This strategy relies on the microbial-induced carbonate precipitation process. Most bacteria can produce or induce the formation of calcium carbonate under suitable conditions [2]. This biogenic precipitation is natural, environmentally friendly, durable and compatible with building materials. Due to these distinct features, microbial $CaCO_3$ has gained more and more attention from researchers and engineers and is being widely investigated in civil engineering, specifically for surface protection [3–9], cementation and consolidation of loose particles [10–14], and crack repair [15–21].

In order to obtain microbial based self-healing, carbonate precipitating bacteria are added into concrete during the mixing process. When cracks appear, bacteria in the crack zone are expected to be activated and precipitate $CaCO_3$ to in-situ heal the cracks. Therefore, the bacteria used should survive the mixing process, remain viable but not active inside the concrete, and become active to precipitate $CaCO_3$ when cracks appear. Also, it is known that the cement-based matrix gradually becomes a denser structure because of the ongoing hydration. Most pores have a size less than 0.5 μm; while the size of bacteria is in the range of 1–3 μm and the size of the spores is around 1 μm. Hence, there is a chance that bacteria would be squeezed and crushed when the pores become smaller. To solve this problem, encapsulation of bacteria before the addition is preferable. The encapsulation material should have a 'shell' function to protect bacteria and have no hindering effect on bacterial carbonate precipitation when cracking occurs. Furthermore, it should be noted that for a realistic self-healing,

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no additional human interference should be required. Because water is an essential element for bacterial activities, obtaining sufficient water with the lowest amount of interference is of crucial importance for realistic self-healing. So far, full submersion has been mostly used in lab tests, which is not feasible in many practical cases.

Regarding the above-mentioned requirements, we applied hydrogel as the bacterial carrier in this research. Hydrogels are hydrophilic gels which have high water absorption and retention capacity. They are widely used in many fields, such as hygienic (especially in disposable diapers), agriculture (water retention in soil, controlled release of fertilizers, etc.) and pharmaceuticals. Hydrogels can also be used to immobilize cells due to their good biocompatibility and mass transport (nutrients and oxygen) properties [22–24]. Therefore, on one hand, the hydrogel can protect the bacteria during the mixing and hydration processes; on the other hand, the swollen hydrogel (water is absorbed from the surroundings after cracks appear) can be used as the water reservoir to support bacterial activities, hence facilitating the formation of the microbial $CaCO_3$.

So far, the healing efficiency in the microbial-based system has been evaluated either directly by visualization of crack filling by light microscopy, or indirectly by measurement of the improvement in matrix properties (strength regain, water-tightness, etc.) after healing [15,17–21,25]. The healing ratio, which was defined as the ratio between the healed crack width and the initial crack width, is often used as a measure. Since the crack width varies along the length, several crack locations are investigated per crack (e.g. at intervals of 0.4 mm in [19]). This is a first possibility for direct quantitative evaluation of the healing efficiency in bacteria based self-healing systems; in fact, it is a semi-quantitative evaluation because only part of the crack locations are investigated and the microscopy analysis is only focused on the surface. Nevertheless, an estimate of the overall self-healing efficiency can still be obtained from this method.

Essentially, healing efficiency relies on the amount and distribution of the healing products formed. Therefore, direct quantification of the total amount and the distribution of the precipitates in the whole specimen is of utmost significance. In this study, high resolution X-ray computed microtomography (X-ray μCT) was used for this aim. X-ray μCT is a non-destructive technique, which generates three-dimensional (3D) images by combining a series of cross-sectional images. μCT analysis is based on measurements of the attenuation of X-rays from different positions of an object and depends on the atomic number and density of the object [26]. It provides information (visualization and quantification) about the internal structure of the matrix without sample preparation or chemical treatment. Recently, X-ray μCT has become a frequently used technique in materials research [27–30]. The obtained resolution is of key importance to get high quality images. The X-ray beam geometry used in laboratories is commonly conical and by moving the sample between the source and the detector, one can choose an appropriate magnification. This has as a consequence that larger objects will have a lower resolution and smaller samples a higher resolution. In order to evaluate the impact of self-healing products inside mortar samples on a pore-scale level, mm-sized samples were investigated in this study.

The aim of this research was to demonstrate the feasibility of applying hydrogel immobilized carbonate precipitating bacterial spores to approach a realistic self-healing in concrete. The self-healing efficiency of the mortar specimens with and without hydrogel encapsulated bacteria incorporated, was investigated both by light microscopy (semi-quantification) and 3D X-ray μCT (full quantification) to quantify the amount of precipitates.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Bacterial strain

The bacterial strain used in this research was *Bacillus sphaericus* LMG 22257 (Belgian Coordinated Collection of Microorganisms, Ghent). Cultivation of *B. sphaericus* spores was performed in MBS liquid medium [31], which contained $MgSO_4{\cdot}7H_2O$ (0.3 g/L), $MnSO_4$ (0.02 g/L), $Fe_2(SO_4)_3$ (0.02 g/L), $ZnSO_4{\cdot}7H_2O$ (0.02 g/L), $CaCl_2$ (0.2 g/L), Tryptose (10 g/L) and Yeast extract (2 g/L). The pH was 7.4 (if not, it was adjusted to a pH of 7.4 by using 1 M HCl or NaOH). Mature spores suspensions ($10^9$ spores/mL) were used as inoculum (1% by volume). The cultures were incubated at 28 °C on a shaker at 100 rpm for 14–28 days until more than 90% of the cells were spores. The spores were then harvested by centrifuging the culture (7000 rpm, 4 °C, Eppendorf MiniSpin, Hamburg, Germany) for 7 min. The centrifuged spores were resuspended in a sterile saline solution (8.5 g/L NaCl). Subsequently, the suspension of the spores was subjected to pasteurization to minimize the amount of vegetative cells in the culture. The final concentration of the spores in the suspension was about $10^9$ spores/mL.

#### 2.1.2. Hydrogel

The hydrogel used was developed by the Polymer Chemistry and Biomaterials Group of Ghent University (PBM-UGent). Commercial Pluronic®F-127 (Sigma Aldrich) was used which is a triblock polymer of poly (ethylene oxide) and poly (propylene oxide) (i.e. PEO–PPO–PEO) and has molecular weight approximately 12,500 daltons. The OH-groups at the end of the chain were modified with methacrylate groups to create double bonds at the end and Pluronic®F-127 bis-methacrylate (Pluronic®-BMA) was obtained. Upon UV irradiation, the photoinitiator Irgacure®2959 (2-dimethoxy-2-phenyl-acetophenone, 224.3 g/mol) will form free radicals which then initiate the polymerization by reacting with the double bonds of Pluronic®-BMA [32]. Finally, a crosslinked polymer network was formed for water absorption and retention inside.

### 2.2. Methods

#### 2.2.1. Encapsulation of the bacterial spores into hydrogel

The bacterial spores were encapsulated into the hydrogel during the process of crosslinking. The suspension of the spores was first mixed with the 20% w/w polymer solution (Pluronic®-BMA). Then, the initiator was also added to the solution. The whole mixture was degassed and mixed for 5 min, and was subjected to UV radiation for 1 h after which a gel sheet formed. For each hydrogel sheet, 10 g polymer solution and 173.8 μL Irgacure 2959 solution (8 g/L) were used. 1 mL spores suspension ($10^9$ spores/mL) was encapsulated in one hydrogel sheet. Hydrogels with or without encapsulated bacterial spores will be represented as HS or H, respectively.

The hydrogel sheets were then subjected to freeze grinding (IKA Yellowline A10 Analytical Grinder) and freeze drying (ChristAlpha 2-4 LSC, Germany) to obtain the dry powders.

#### 2.2.2. Mortar specimens

Mortar specimens were made by using cement (CEM I 52.5N), sand (DIN EN 196-1 Norm Sand) and tap water. The water to cement ratio (*w/c*) by mass was 0.5 and the sand to cement ratio by mass was 3. Four series of specimens were made. Group R are specimens without any additions. Group N are specimens with all the nutrients added, including the food for bacteria (yeast extract) and the deposition agents (urea and $Ca(NO_3)_2{\cdot}4H_2O$).