EFFECT OF WATER AVAILABILITY ON MICROBIAL SELF-HEALING OF CONCRETE

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ABSTRACT

Microbial-based self-healing is a promising solution for a sustainable concrete. The principle is that carbonate precipitating bacteria and bio-reagents are pre-added into concrete; upon cracking, bacteria will be activated to precipitate $CaCO_3$ to heal the crack. Due to the harsh environment in concrete, encapsulation of bacteria is preferable before incorporation into the matrix. In this study, microcapsules and hydrogels were applied to encapsulate bacterial spores.

Water is an essential element for bacterial activities. Therefore, water availability is a key factor to obtain considerable amount of healing. In this work, self-healing behavior of the specimens (with and without bacteria incorporated) were investigated at the conditions of 95%RH and wet-dry cycles (wdc). Two types of wdc were applied: 1) 8h in water and 16h in 60%RH; 2) 1h in water and 11h in 60%RH. Healing efficiency was evaluated by crack closure and the decrease of water permeability. No crack healing was visualized in the specimens stored at 95%RH. The specimens subjected to wdcs had much more crack healing. Water permeability of the specimens with microencapsulated bacteria was about one order of magnitude lower than that of the reference ones. About 40% and 80% of the crack area was healed in the reference and bacterial specimens (at wdc1), respectively. For the ones stored at wdc2, though with a reduced wet period, considerable amount of healing was obtained in the specimens with hydrogel encapsulated bacteria, which had crack healing ratio of 5%~30% and 50%~100% for the reference and bacterial series, respectively. This study indicates that incorporation of carbonate precipitating bacteria into concrete can enhance the self-healing efficiency. The amount of available water and the time during which the water can stay available in cracks are of crucial importance to obtain a sufficient healing. This water availability can be enhanced by encapsulating the bacteria in hydrogels.

KEYWORDS: self-healing, crack, bacteria, $CaCO_3$, available water

INTRODUCTION

Crack formation is a big concern in concrete. It is the start and also a powerful accelerator of concrete deterioration. Although in most cases, early age micro-cracks do not affect the load-carrying capacity of the concrete structure, they can still affect its transport properties, and hence durability. The reason is that cracks provide an easy access to water, gases and other aggressive agents (chloride, sulfate, etc.). Repair and rehabilitation of deteriorated concrete structures are of significant importance because they cannot only extend the service life of the structure but also assure the safety today and in the future. So far, the conventional repairing techniques have gradually developed into a sound repairing system and achieved very good effect. Only, it implies large costs, of both money and time, for the regular inspection, monitoring, maintenance and repair, since cracks in concrete can occur in any stage of the service life due to non-ideal service environment.

An alternative solution is self-healing and self-repairing. Like in the bio-systems, animals and trees usually can heal small body damage by themselves without any other intervention, concrete also has a potential of intrinsic self-healing, which is named autogenous healing. The main mechanisms relate with further hydration of unhydrated cement particles and precipitation of calcium carbonate ($CaCO_3$) due to the dissolution of calcium hydroxide ($Ca(OH)_2$). Besides, swelling of CSH also donates volume for a decrease of crack width [1]. Autogenous healing is influenced by a lot of factors including environmental factors and the concrete composition itself. So it is quite difficult to obtain a predictable, reliable and controllable healing efficiency.

In order to achieve more healing efficiency with certainty, different strategies are investigated with the aim of autonomous self-healing of concrete cracks. One promising strategy is microbial based self-healing. Microorganisms, especially bacteria, have a geochemical activity which is responsible for the deposition of minerals to a great extent throughout the history of the Earth. They can induce or control the precipitation of different kinds of minerals, like oxides, sulphates, phosphates, carbonates, etc. [2]. Carbonate precipitation induced by bacteria has been regarded as environmentally friendly and economical material which has a promising potential for wide engineering applications.

It can be seen that water is important both for autogenous healing and bacterial based healing. The reactions such as secondary hydration of unhydrated cement, precipitation $CaCO_3$ from $Ca(OH)_2$ and CO_2, and bacterial activities (water is the major factor preventing or limiting microbial growth), etc, all need water. So far, most of the microbial based self-healing was performed under the condition of full submersion to obtain sufficient water for crack healing [3-4]. However, full immersion is difficult to achieve in many practical situations. In this study, two wet-dry cycle regimes were used to mimic more realistic situations (alternated wetting by rain and drying). The effect of water availability in these two conditions for microbial crack healing was investigated.

MATERIALS AND METHODS

Microbial source

Bacillus sphaericus LMG 22557 (Belgian coordinated collections of microorganisms, Ghent) was used in this study. It is an alkali-tolerant spore-forming strain. The remarkable characteristic of this strain is its high productivity of calcium carbonate precipitation by the pathway of urea hydrolysis [5]. The cultivation of *B. sphaericus* spores occurred in the liquid minimal basal salts (MBS) medium [6]. Mature spores were transferred as inocula (1% v/v) into MBS medium. The cultures were incubated (28 ˚C, 100 rpm) for 14~28 days until more than 90% of the cells were spores. The spores were harvested by centrifuging the culture for 7min. The centrifuged spores were resuspended in the sterile saline solution (NaCl, 8.5g/L). Subsequently, the suspension of the spores was subjected to pasteurization to minimize the amount of vegetative cells in the culture. The concentration of the spores in the suspension was about 10^9 spores/mL, which was stored in a 4°C fridge for further use.

Self-healing in mortar specimens with microencapsulated spores

Encapsulation

The microcapsules were melamine based and contained inert substance to protect the bacterial spores. The bacterial spores were encapsulated following a patented poly-condensation reaction based microencapsulation process by the company Devan Chemicals NV (Patent WO 2010/142401). The average diameter of the microcapsules was about 5μm. After encapsulation, an emulsion consisting of microencapsulated spores and water was obtained. The concentration of the spores in the microcapsules was around 10^9 cells/g microcapsules (dry weight).

Preparation of mortar specimens

A series of mortar specimens was made with a water to cement ratio of 0.5 and sand to cement ratio of 3 by using ordinary Portland cement CEM I 52.5N and standard sand (DIN EN 196-1). The detailed composition is shown in Table 1. Group N are the specimens with all nutrients needed for bio-precipitation. Group NC and NCS are the specimens with nutrients and microcapsules which did not (NC) or did (NCS) contain spores. Two types of specimens were made: prisms (30 mm x 30 mm x 360 mm) with a reinforcement (Φ = 6 mm, L = 660 mm) in the center and cylinders (Φ = 78 mm, H = 22 mm). To make cylinders, firstly, a mortar layer (about 10mm) was brought into the moulds. After vibration, two steel fibers (Φ = 1 mm, L = 50 mm) were put on top of the layer. Subsequently, the moulds were completely filled with mortar and vibrated. After casting, the moulds were put in an air-conditioned room (20°C, > 95%RH) for 24h. The specimens were then demoulded and placed in the same room.

Table 1 Composition of the specimens in each series

	Cement (g)	Sand (g)	Water (g)	Nutrients (g)	Microcapsule emulsion (g)	Dry weight of microcapsules (g)	Bacterial spores
R	450	1350	225	0	0	0	N
N	450	1350	214	57.84	0	0	N
NC	450	1350	201.4	57.84	26.1	13.5	N
NCS	450	1350	192.8	57.84	34.7	13.5	Y

Nutrients included yeast extract, urea and $Ca(NO_3)_2.4H_2O$. The addition dosage was 0.85%, 4% and 8% of cement by weight. $Ca(NO_3)_2.4H_2O$ contains 30.5wt% water. Therefore, the amount of mixing water was reduced by the amount of water in $Ca(NO_3)_2.4H_2O$ and the amount of water in microcapsule emulsions. The last column shows whether bacteria were present (Y) or not (N).

Crack creation and specimens incubation

Cracks were created in the specimens 28 days after casting. The prisms were subjected to a tensile test to create multiple cracks. A uniaxial tensile load was applied to the specimen at a speed of 0.01mm/s under stroke control. The loading was stopped when the average crack width reached 150 μm. The cylinders were subjected to a splitting test to create a single crack in the center of the specimen. The crack width was controlled by the attached linear variable differential transformer (LVDT). The final crack width in the cylinders was in the range 0.20-0.22 mm.
The cracked specimens were subjected to two kinds of incubation conditions for 8 weeks: 1) 20°C, > 95%RH; 2) wet-dry cycles (wdc 1). During one wet-dry cycle, the specimens were immersed in water for 16h and then exposed to air (20°C, 60%RH) for 8h.

Evaluation of self-healing effect

The self-healing efficiency was evaluated by crack filling and water tightness measurements.
Crack filling in prisms was investigated by means of light microscope (Leica S8 APO, Switzerland). Images of the cracks were taken before and after incubation; and crack area was analyzed by a Leica image analysis program. Healing ratio, which was the ratio between healed crack area and initial crack area, was calculated and used to quantify the filling effect.
Cracked cylinders were subjected to a low pressure water permeability test to investigate the water tightness after incubation (n=3). The detailed description of the test process and the calculation of the water permeability coefficient (k values) can be seen in previous research [7].

Self-healing in mortar specimens with hydrogel encapsulated spores

Encapsulation

The hydrogel was developed by the Polymer Chemistry and Biomaterials Group of Ghent University (PBM-UGent). It was synthesized based on Pluronic®F-127 bis-methacrylate (Pluronic®-BMA) as the monomer and Irgacure 2959 as the photoinitiator for free radical polymerization. Bacterial spores

suspension (10^9 cells/mL) was first mixed with 20% w/w Pluronic®F-BMA solution. Then the initiator was also added to the solution. The mixture was mixed and degassed for 5min, and subjected to UV radiation for 1h. A hydrogel sheet was then 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), 0.4g yeast extract and 0.9g urea were encapsulated in one hydrogel sheet. The obtained hydrogel sheets were subjected to freeze grinding and freeze drying to obtain the dry powders. The hydrogels with or without spores loaded were represented as HS and H, respectively.

Preparation of mortar specimens

Similarly, four series of mortar specimens were made with a water to cement ratio of 0.5 and sand to cement ratio of 3 by using ordinary Portland cement CEM I 52.5N and standard sand (DIN EN 196-1). The detailed composition is shown in Table 2. Group NH and NHS are the specimens with nutrients and hydrogels which were not (NH) or were (NHS) loaded with spores.

Table 2 Composition of the specimens in each series

	Cement (g)	Sand (g)	Water (g)	Nutrients (g)	Dry weight of hydrogels (g)	Bacterial spores
R	450	1350	225	0	0	N
N	450	1350	214	57.84	0	N
NH	450	1350	214	57.84	22.5	N
NHS	450	1350	214	57.84	22.5	Y

The composition of the nutrients was the same as mentioned in Table 1.

The same types of specimens were made as the ones with microcapsules. After casting, the moulds were put in the same air-conditioned room (20°C, > 95%RH) for 24h. The specimens were then demoulded and placed in the same room.

Crack creation and specimens incubation

The methodologies to create cracks in prisms and cylinders were the same as those for the specimens with microcapsules. The cracked specimens were subjected to two kinds of incubation conditions for 4 weeks: 1) 20°C, > 95%RH; 2) wet-dry cycles (wdc 2). During one wet-dry cycle, the specimens were immersed in water for 1h and then exposed to air (20°C, 60%RH) for 11h.

Evaluation of self-healing effect

The self-healing efficiency was also evaluated by crack filling and water tightness measurements. Crack width changes before and after healing were analyzed. While healing ratio, which is the ratio between healed crack width and initial crack width, was calculated and used to quantify the filling effect. The same water permeability tests (n=3) were performed twice, before and after the incubation. The decreased water permeability was used to evaluate the healing effect.

RESULTS AND DISCUSSION

Self-healing behaviour in the specimens with microencapsulated spores

Crack filling in the specimens stored at different storage conditions

An overview of the total initial, total final, cumulative healed crack area, and the healing ratios in the specimens stored in air and in wet dry cycles is shown in Figure 1. It can be seen that the initial crack area was different in different specimens though the same methodology was used to create cracks. Therefore, both the absolute amount of healed crack area and healing ratio were used to evaluate the filling efficiency.

No obvious crack healing was observed in the specimens which were stored at 95%RH. Both healed crack area and healing ratios were almost zero. While for the ones in wet-dry cycles, the amount of crack area was decreased after incubation. The healed crack area was in the range of 12 mm^2 to 81 mm^2 and the healing ratio was from 40% to 80%. The healing in the R specimen was due to autogenous healing. The addition of nutrients had a positive effect on autogenous healing, resulting in an increased amount of healed crack area and healing ratio in the specimens N and NC, compared to the specimen R. The reason could be that the added Ca-nitrate provided more available Ca^{2+} for precipitation of $CaCO_3$. The highest healing efficiency was obtained in the specimen with microencapsulated spores incorporated (NCS), which had a healed crack area of about 81 mm^2 and healing ratio of 80%. The greatly enhanced healing efficiency (at least 50% higher than other specimens) was due to bacterial $CaCO_3$ precipitation.

Both autogenic and biogenic crack healing need free water. Crack healing can only take place when specimens are thoroughly wet. This is why no obvious healing happened even in a high humidity environment (95%RH). Although without the same amount of available water as in full immersion, wet-dry cycles can still provide sufficient water for crack healing. During the wetting period, the specimens were immersed in water and absorbed sufficient water in the matrix. When they were exposed to the air (dry period), the absorbed water could still keep the matrix in wet state for some time because the water will evaporate gradually. The CO_2 dissolved in the surface films of water is available for reacting with $Ca(OH)_2$, and hence, $CaCO_3$ can be formed. Besides, the dissolved oxygen and nutrients in the surroundings facilitate the precipitation of biogenic $CaCO_3$ in the specimens with bacteria embedded. Due to this two-fold precipitation of $CaCO_3$ (abiotic-$CaCO_3$ and biogenic-$CaCO_3$), the crack filling effect in bacterial series was much higher than for non-bacterial series.

It is assumed that for the specimens with bacteria, a reduced immersion time can also decrease the escape of the bacteria from the crack surface and the leakage of the nutrients into the incubation solution. An optimal wet-dry cycle can promote the diffusion of the nutrients from the internal matrix to the surficial cracking zone without leaching too much to the bulk solution and can keep the specimens with sufficient available water for bacterial activities during the dry state.

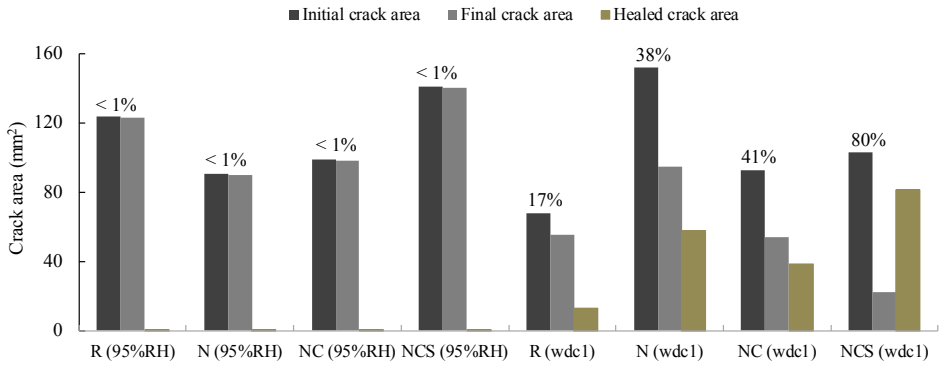

Figure 1 – Crack filling in the specimens stored in air and in wdc1 (the values on the bars indicated crack healing ratio)

Water permeability

Because no crack healing was observed in the specimens stored at 95%RH, only the cylinders in wet-dry cycles were subjected to the water permeability test. During the 30 days testing, it was noticed that the water permeability coefficient k gradually reached a stable value, especially the k values after 15 days were almost stable. And the difference in the k-value between day 0 and day 30 was quite small. Therefore, the final water permeability of the specimens was represented by the average k-value from day 15 to 30, which is shown in Figure 2 (three dots represent three replicates). The k values in the R specimens were in the range of 10^{-5} m/s to 10^{-6} m/s. The N specimens had a slightly lower water

permeability, around 10^{-6} m/s. Compared with R and N, the series with microcapsules incorporated (NC and NCS) had generally a lower permeability. Although big variations occurred within NCS series, it still had the lowest water permeability, in the range of $4*10^{-7}$ m/s to $3*10^{-9}$ m/s. The water permeability test results confirmed that self-healing efficiency, in the aspect of water tightness, was greatly improved by use of microencapsulated spores.

It should be noted that there were some variations in k values within the same series, especially the bacterial series. This is attributed to the fact that autogenous healing and bacterial based healing could not be homogenous along the whole crack. Therefore, the crack filling effect was different even in the same kind of specimens. Besides, the initial crack widths cannot be exactly the same in all specimens, contributing to different water permeability which is greatly dependent on crack width. Thus, it can be seen that in a bacterial based self-healing system, multiple crack healing efficiency is much more representative than single crack healing efficiency.

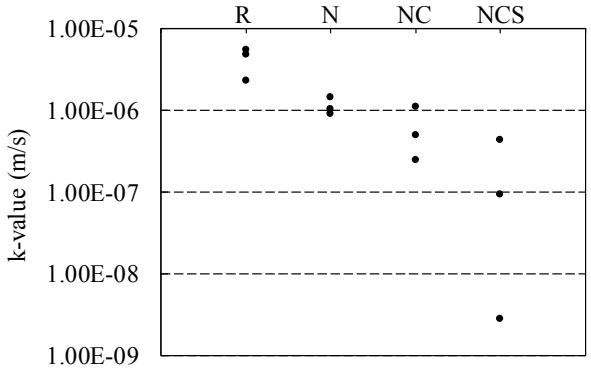

Figure 2 – Water permeability of the specimens after storage in wdc1

Self-healing behaviour in the specimens with hydrogel encapsulated spores

Crack filling efficiency

Average crack healing ratio based on crack width changes (in different crack width ranges) was used to evaluate multiple crack healing efficiency in this part.

Similarly, all the specimens stored at 95%RH, including the reference ones and the ones with hydrogels (with or without bacterial spores incorporated), showed no obvious crack filling. But the ones incubated in wet-dry cycles, all showed crack healing. The healing ratios in different series can be seen in Figure 3. The average crack healing ratio in non-hydrogel series, hydrogel and bio-hydrogel series was in the range of 5%-30%, 25%-50% and 50%-100%, respectively. The specimens with hydrogel added, especially the ones with bacteria loaded hydrogels, exhibited an significant superiority in crack healing. Crack healing ratio was about 80%-100% and 50% for the cracks in the range of 0~0.3mm and 0.3~0.5mm, respectively. The improved crack filling efficiency was due to bacterial $CaCO_3$ precipitation.

It can be seen that for the same R and N specimens, the crack filling efficiency was much lower in wdc2 than that in wdc1. This is attributed to the fact that the wetting period in wdc2 was much shorter than that in wdc1, 2h per 24h versus 16h per 24h. This means that the absorbed water will evaporate within a short time and the specimens are mainly in a dry state when they were exposed to the air, resulting in limited amount of crack healing. Hydrogels can not only absorb water in the wetting stage but also retain the absorbed water when exposed to air. Therefore, there was sufficient available water provided for continuous autogenous healing and bacterial activities (bio-precipitation) during the whole wet-dry periods, and hence, more crack healing can be expected in the specimens NH and NHS. Due to the precipitation of abiotic-$CaCO_3$ and biogenic-$CaCO_3$, the crack filling effect in bacterial series was much higher than in non-bacterial series.

The wet-dry interval in this study was determined by the water retention capacity of the hydrogel. It was found that the hydrogel can keep the absorbed water for 48h in the air (20°C, 60%RH). In the first 12h, about 70% of the available water stayed inside the hydrogels (data not shown). Therefore, the wet-dry interval was designed as 1h in water and 11 h in air to make sure that there was enough available water still in the hydrogels when exposed to air.

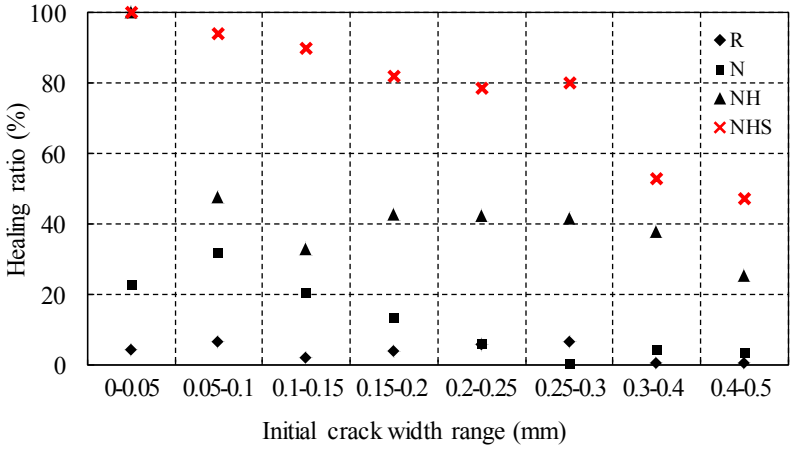

Figure 3 – Crack filling in the specimens stored in air and in wdc2

Water permeability

Only the cylinders stored at wet-dry cycles were subjected to the water permeability test. It should be noted that the properties of the mortar specimens (strength, microstructure, etc.) were greatly changed by hydrogel addition (data not shown). The water permeability of the matrix itself therefore was not comparable anymore among different kinds of specimens. Therefore, the water permeability test was performed before and after the wet-dry cycles to compare the difference (decrease) of the k-values.
In previous experiments, the water permeability test lasted around 30 days and one measurement was done per day. The k-values gradually became stable after 15 days and the final k-value was expressed as the average of the stable values. However, to test the initial k-value, it is not appropriate to perform the test for 30d because the specimens will be always immersed in the water of the setup, which will trigger considerable autogenous healing during this long test period. Bacterial spores will also germinate and precipitate $CaCO_3$. Therefore, the test period for initial k-value should not last more than 1d. Alternatively, 15 k-values were measured continuously within one day after the test was started. The average value from the stable measurements was used as the water permeability coefficient for the specimens. After the test finished, the specimens were taken out from the setup and subjected to the wet-dry cycles. The final k-value was tested again following the same methodology after 4 weeks.

The initial and final k-values of different specimens are shown in Figure 4. It can be seen that the initial k-values of the specimens with hydrogels (NH and NHS) were much higher than those of the specimens without hydrogels (R and N), indicating a more porous structure. After wet-dry cycles, crack healing occurred, resulting in the decrease of the k-values. Due to limited amount of crack-healing in R and N specimens, the decrease of the k-values was not so profound compared to that of the specimens with (bio-)hydrogels incorporated. Nevertheless, the final k-values in hydrogels series were still higher than in non-hydrogel series. The k-values were decreased by 48% to 75% in NH and 41% to 77% in NHS. No significant difference in decreased water permeability was found between NH and NHS, though the specimen NHS had considerable higher multiple crack healing efficiency than that of the specimen NH.

Due to the addition of hydrogels, the specimens became very porous and the strength was greatly decreased (data not shown), which made it difficult to create cracks at the same crack widths. Besides, some crack walls were connected with pores, which caused extra crack volume; and the crack width

varied a lot along the whole crack. The influence from irregular crack geometry and porous matrix on water permeability could be more than the influence from crack healing itself. Therefore, it is hard to conclude whether the specimen NHS had a better crack healing than the specimen NH or not, regarding the aspect of water tightness.

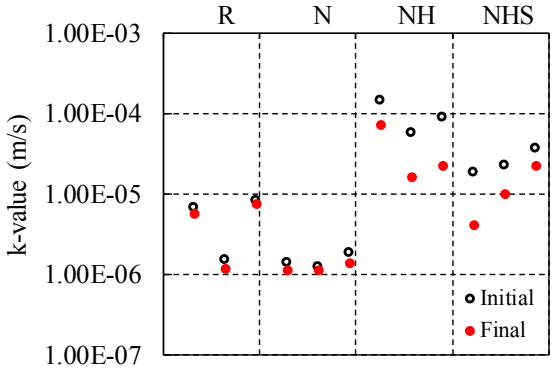

Figure 4 – Water permeability of the specimens before and after storage at wdc2

CONCLUSIONS

In this study, it was confirmed that free water is an essential element for crack healing. Without free water, even in a high humidity condition, almost no autogenous healing or microbial-based healing occurred. Both microencapsulated spores and hydrogel encapsulated spores can greatly increase the crack healing efficiency. Full immersion is not an essential condition since a suitable wet-dry cycle can also provide sufficient available water. Due to the water absorption and retention capacity of the hydrogel, considerable crack filling can still be obtained in the surroundings with less amount of water provided. However, the drawback of hydrogel that it declines the properties of the matrix cannot be neglected and needs to be solved in further research.

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