

FUNCTIONALLY GRADED BACTERIA CAPSULES FOR CONCRETE SELF-HEALING

Cross-Reference to Related Application

5 [0001] This application claims the benefit of priority of Singapore Patent Application No. 10202103685S, filed 12 April 2021, the content of it being hereby incorporated by reference in its entirety for all purposes.

Technical Field

10 [0002] The present disclosure relates to a capsule incorporable into a cement-based material for repairing a crack in the cement-based material. The present disclosure relates to a nutrient capsule incorporable into a cement-based material for housing a nutrient which aids a bacterial spore for repairing a crack in the cement-based material. The present disclosure relates to a cement-based material incorporated with (i) the
15 capsule, or (ii) the capsule and the nutrient capsule. The present disclosure also relates to a method of forming the capsule, the nutrient capsule and the cement-based material.

Background

[0003] Repairing of cracks is crucial for concrete structures as the formation of cracks
20 allows the ingress of aggressive substances, leading to loss of durability and shortened service life. However, traditionally used manual repair methods are labor-intensive, inconvenient and costly. It was reported that the estimated annual cost of concrete repair is 18-21 billion dollars in the United States of America alone. To avoid these drawbacks of manual repair, bacteria-based self-healing techniques were
25 investigated to realize autogenous crack healing in Portland cement (PC). This method involves embedding dormant bacteria spores together with compounds (e.g. such as nutrients and calcium source) directly into the concrete mix during the casting process. When cracks propagate, the bacteria spores are exposed and activated with the ingress of water and oxygen, leading to precipitation of carbonate phases within the
30 cracks through the metabolic activities of the bacteria.

[0004] However, studies have shown the lack of precipitation when the bacteria were directly embedded in PC (i.e. without any protection) for 28 days, indicating the

inability of bacteria to realize self-healing in PC in such instances. This inactivity of bacteria may usually be associated with the high pH of PC (pH is around 13), resulting in the development of encapsulation methods to protect bacteria from unfavourable surroundings. Traditionally, polymers (e.g. melamine and hydrogel), porous carriers (e.g. expanded clay, expanded perlite and fibers) and low-alkalinity cementitious materials which mainly gain strength through hydration may be most widely used materials for the encapsulation. Although these encapsulations may prolong the life of bacteria, there are limitations arising from use of these materials. Extensive studies have reported that the use of polymers as capsule tends to result in significant strength reduction to the matrix, which is mainly associated with two reasons. First, polymers are soft materials with lower strength than cementitious materials, rendering a weak point in the system. Second, introduction of the polymer capsule tends to create larger pores inside the matrix, and the higher porosity led to strength decrease of the concrete. As for the use of porous materials to carry bacteria inside, the high porosity of these materials does not provide a full protection for bacteria. After embedding bacteria into these porous carriers by soaking or vacuum impregnation, bacteria can still be in contact with the surrounding environment during mixing. As the porous structure of the carrier allows the penetration of the bacteria inside, it also allows the penetration of the alkaline compositions from the binder component which hinders the bacteria from “healing” the concrete. As for the use of hydration-based cementitious materials, the continuous hydration of the cement tends to result in continuous densification of the microstructure of the capsules. As a result, bacteria are potentially crushed due to the insufficient space. Besides, the capsule shows a rather uniform density over the whole capsule, which may render some difficulties in bacteria exposure when cracks propagate.

[0005] There is thus a need to provide for a solution that addresses one or more of the limitations mentioned above.

Summary

[0006] In a first aspect, there is provided for a capsule incorporable into a cement-based material for repairing a crack in the cement-based material, the capsule comprising:

- an inorganic particle having a surface and a core region,
- wherein the core region houses a bacterial spore for repairing the crack;

or

wherein the core region houses a bacterial spore for repairing the crack and the capsule houses a nutrient separate from the bacterial spore;

wherein the inorganic particle has a density and a strength which decrease in a direction from the surface to the core region, and

wherein the density at the surface sufficiently hinders penetration of an alkaline substance.

[0007] In another aspect, there is provided for a method of forming the capsule described in various embodiments of the first aspect, the method comprising:

providing a bacterial spore for repairing a crack in a cement-based material;
forming a paste comprising the bacterial spore and water or forming a paste comprising the bacterial spore, water and a nutrient;
allowing the paste to harden;
converting the paste which has hardened into particles; and
curing the particles in the presence of carbon dioxide to form the capsule.

[0008] In another aspect, there is provided for a nutrient capsule incorporable into a cement-based material for housing a nutrient which aids a bacterial spore for repairing a crack in the cement-based material, the nutrient capsule comprising:

an inorganic particle having a surface and a core region,
wherein the inorganic particle has a density and a strength which decrease in a direction from the surface to the core region, and
wherein the core region houses the nutrient and the capsule is absent of a bacterial spore.

5 **[0009]** In another aspect, there is provided for a method of forming the nutrient capsule as described in various embodiments herein, the method comprising:

providing a nutrient which aids a bacterial spore for repairing a crack in a cement-based material;
forming a paste comprising the nutrient and water;
10 allowing the paste to harden;
converting the paste which has hardened into particles; and
curing the particles in the presence of carbon dioxide to form the nutrient capsule.

[0010] In another aspect, there is provided for a cement-based material incorporated with (i) the capsule described in various embodiments of the first aspect, or (ii) the capsule described in various embodiments of the first aspect and the nutrient capsule described in various embodiments herein.

[0011] In another aspect, there is provided a method of forming the cement-based material as described in various embodiments herein, the method comprising:

providing (i) a capsule described in various embodiments of the first aspect, or (ii) a capsule described in various embodiments of the first aspect and a nutrient capsule
5 described in various embodiments herein; and

mixing the capsule, and when present, the nutrient capsule, with a cement to form the cement-based material.

Brief Description of the Drawings

10 [0012] The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the present disclosure. In the following description, various embodiments of the present disclosure are described with reference to the following drawings, in which:

[0013] FIG. 1A shows a servo-hydraulic universal testing machine for crack generation
15 for self-healing samples (e.g. cylindrical specimens).

[0014] FIG. 1B shows pre-cracked specimens with marks.

[0015] FIG. 2 shows a X-ray diffraction (XRD) pattern of a capsule of the present disclosure, containing 20 wt% fluorite.

[0016] FIG. 3 shows SEM images of two different RMC-based capsules of the present
20 disclosure, which are fabricated by the method of the present disclosure. The SEM images demonstrate that the two different RMC-based capsules have uniform morphology.

[0017] FIG. 4A shows microscope image before and after self-healing of a crack using sample from group P.

25 [0018] FIG. 4B shows microscope image before and after self-healing of a crack using sample from group PNB.

[0019] FIG. 4C shows microscope image before and after self-healing of a crack using sample from group PNC.

[0020] FIG. 5 is a plot of 28-day compressive strength of P, PNC, PN and PC* samples.

[0021] FIG. 6 shows particle size distribution of the reactive magnesia cement-based bacteria spores (RMC-B) capsules of the present disclosure.

5 [0022] FIG. 7 shows the procedure to quantify the bacteria viability in the RMC-B and the PC-B capsules.

[0023] FIG. 8A shows a steel mesh placed in the half-full mold for self-healing specimen preparation.

[0024] FIG. 8B shows molds fully filled for self-healing specimen preparation.

[0025] FIG. 9 shows a water passing rate measurement setup.

10 [0026] FIG. 10A is a sketch of sample extraction for the examination of healing products under SEM to demonstrate surface healing.

[0027] FIG. 10B is a sketch of sample extraction for the examination of healing products under SEM to demonstrate internal healing.

15 [0028] FIG. 10C is a sketch of sample extraction for the examination of healing products under SEM to demonstrate precipitation on specimen surface.

[0029] FIG. 11 shows SEM images of the RMC-B capsules of an example of the present disclosure.

[0030] FIG. 12 is a plot of weight loss of raw RMC material.

20 [0031] FIG. 13 is a schematic diagram of the gradient structure of the RMC-B capsule with dense shell and porous core.

[0032] FIG. 14 is a plot of the viability of bacteria spores in PC-B capsules.

[0033] FIG. 15 is a plot of the viability of bacteria spores in RMC-B capsules.

[0034] FIG. 16 shows the isothermal calorimetry results of the Mix P and Mix P-C of an example of present disclosure.

25 [0035] FIG. 17 is a plot of compressive strength of PC paste with and without the addition of RMC-B capsules.

[0036] FIG. 18 shows plots of the crack widths of different specimens before and after 5 wet/dry cycles.

30 [0037] FIG. 19A shows optical microscope images of a crack in sample PNC before and after 5 wet/dry conditioning cycles.

[0038] FIG. 19B shows optical microscope images of a crack in sample PNS before and after 5 wet/dry conditioning cycles.

[0039] FIG. 19C shows optical microscope images of a crack in sample P before and after 5 wet/dry conditioning cycles.

[0040] FIG. 20 shows the water passing rate reduction of samples PNC, PNS and P after 5 wet/dry conditioning cycles.

5 [0041] FIG. 21A is a SEM image of the healing products on the crack surface of a PNC specimen.

[0042] FIG. 21B is a SEM image of the healing products on the crack interior of a PNC specimen.

10 [0043] FIG. 21C is a SEM image of the healing products on the surface of a PNC specimen.

[0044] FIG. 22 is a SEM image of the RMC-B-N capsule.

[0045] FIG. 23 shows XRD pattern of the RMC-B-N capsule.

[0046] FIG. 24A is a plot of crack widths of different specimens (P-BNC and P) before and after 10 wet/dry cycles.

15 [0047] FIG. 24B is a plot of crack widths of different specimens (P-BNC and P) before and after 20 wet/dry cycles.

[0048] FIG. 24C is a plot of crack widths of different specimens (P-BNC and P) before and after 30 wet/dry cycles.

20 [0049] FIG. 25A shows a photo of a crack with width 235 μm from P-BNC group before healing.

[0050] FIG. 25B shows a photo of a crack with a starting width 235 μm from P-BNC group after 10 cycles of conditioning.

[0051] FIG. 25C shows a photo of a crack with a starting width 235 μm from P-BNC group after 20 cycles of conditioning.

25 [0052] FIG. 26 is a plot of the average water passing rate reduction of different specimens at 10, 20 and 30 cycles of conditioning.

[0053] FIG. 27 is a plot of compressive strength of specimens with (P-BNC) and without (P) addition of RMC-B-N capsules.

30 [0054] FIG. 28 is a plot of compressive strength of different specimens with addition of PC-based nutrients capsules and RMC-B capsules.

Detailed Description

[0055] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the present disclosure may be practised.

5 [0056] Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments. Features that are described in the context of an embodiment may correspondingly be applicable to the other embodiments, even if not explicitly described in these other embodiments. Furthermore, additions and/or combinations and/or alternatives as
10 described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

[0057] The present disclosure relates to a capsule incorporable into a cement-based material for repairing a crack in the cement-based material. The capsule can be used as a cement additive to engage in self-healing in concrete and other cement-based
15 materials. The capsule may be termed herein “a functionally graded bacteria capsule”, as the capsule has a density and a strength which decrease in a direction from the surface to a core region of the capsule with a bacterial spore incorporated (confined) in the core region. Said differently, the present capsule differs from traditional capsule discussed in the background section hereinabove as the present capsule has a gradient density and
20 strength, i.e. having a gradual reduction of density and strength from the surface to the core region of the capsule.

[0058] The present disclosure also relates to a method of forming such capsule. To fabricate such capsules, inorganic materials with low alkalinity (e.g. pH of 12 or less, or pH of 11 or less), porous structure, and formable into a particle to have a dense
25 surface, aforesaid density and strength transition, can be used. As non-limiting examples, reactive magnesia cement (RMC) or any RMC-based mixes (e.g. RMC incorporating waste materials/aggregates/additives) can be used to fabricate the present capsule. Taking pure RMC mix as a non-limiting example, RMC is a low alkalinity cementitious material (pH is around 10.5) which mainly gains strength through
30 carbonation. The strength evolving process includes 2 steps. For instance, firstly, MgO hydrates to produce brucite with high porosity and low strength. Brucite then undergoes carbonation and forms a range of hydrated magnesium carbonates (HMCs) such as

nesquehonite ($\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$), hydromagnesite ($4\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$), and dypingite ($4\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 5\text{H}_2\text{O}$), which are dense and strong. Carbonation is mainly a surface reaction, and thus the HMC phases are formed on the surface region. Under accelerated carbonation with high CO_2 concentration, the carbonated surface
5 forms a dense shell which hinders further penetration of CO_2 towards the core region of the capsule such that the core region remains porous as the major phase within the sample are still brucite. Due to this feature of RMC, the present capsule has a gradient density and strength with a gradual reduction of density and strength from the surface area to the core region.

10 **[0059]** The present disclosure also relates to a nutrient capsule incorporable into a cement-based material for housing a nutrient which aids a bacterial spore for repairing a crack in the cement-based material. The present disclosure also relates to a cement-based material incorporated with (i) the capsule, or (ii) the capsule and the nutrient capsule. The present disclosure also relates to a method of forming the capsule, the
15 nutrient capsule and the cement-based material.

[0060] Details of various embodiments of the present capsule, the nutrient capsule, the cement-based material incorporated with (i) the capsule, or (ii) the capsule and the nutrient capsule, and their method of forming, and advantages associated with the various embodiments are now described below. Where an embodiment or an advantage
20 has been described in the examples section further hereinbelow, it shall not be iterated for brevity.

[0061] In the present disclosure, there is provided for a capsule incorporable into a cement-based material for repairing a crack in the cement-based material. The capsule may comprise an inorganic particle having a surface and a core region.

25 **[0062]** In various non-limiting embodiments, the core region houses (i) a bacterial spore for repairing the crack or (ii) the core region houses a bacterial spore for repairing the crack and the capsule houses a nutrient separate from the bacterial spore.

[0063] In various embodiments, the inorganic particle has a density and a strength which decrease in a direction from the surface to the core region, and the density at the
30 surface sufficiently hinders penetration of an alkaline substance. With the capsule having a dense surface, the capsule advantageously protects the bacterial spore therein from any harmful externalities. Structures formed of concrete or cement-based material

may develop cracks over time. Advantageously, the capsule can be incorporated into the concrete or cement-based material at the start prior to forming the structure. Over time, as cracks develop in the concrete or cement-based material, cracks can also develop in the capsule, which in turn exposes the bacterial spore for repairing the cracks.

[0064] In various embodiments, the core region can be porous. The porosity advantageously allows for the bacterial spore to be housed in the capsule away from the surface.

[0065] In various embodiments, the core region may comprise brucite and the surface may comprise hydromagnesite or nesquehonite. The hydromagnesite and nequehonite advantageously imparts strength to the surface of the capsule to better withstand any compressive forces that may damage the bacterial spore.

[0066] In certain non-limiting embodiments, where the core region houses the bacterial spore for repairing the crack, the capsule can be absent of a nutrient.

[0067] In various embodiments, the nutrient may comprise yeast extract. In certain non-limiting embodiments, when the capsule houses the nutrient separate from the bacterial spore, the nutrient may comprise yeast extract.

[0068] In various embodiments, the bacterial spore is a dormant bacteria. In other words, the bacterial spore in the capsule is in a dormant state which can be activated with the nutrient to repair cracks. The bacterial spore, being dormant, is more resistant to externalities (adverse temperature, pH, etc.) compared to its active state, i.e. the vegetative bacteria. Said differently, in the context of the present disclosure, a bacteria in its dormant state is referred to as a bacterial spore while a bacteria in its active state is referred to as a vegetative bacteria. In various embodiments, the bacterial spore may comprise *Bacillus cohnii*, *Bacillus halodurans*, and/or *Bacillus pseudofirmus*.

[0069] In various embodiments, the capsule may further comprise a calcium precursor. A non-limiting example of the calcium precursor may include calcium lactate, calcium acetate, calcium glutamate, and calcium formate. The bacterial spore may render calcium to precipitate from the calcium precursor so that the calcium mineral seals up the cracks.

[0070] The present disclosure provides for a method of forming the capsule described in various embodiments of the first aspect. The method comprises providing a bacterial

spore for repairing a crack in a cement-based material, forming a paste comprising the bacterial spore and water or forming a paste comprising the bacterial spore, water and a nutrient, allowing the paste to harden, converting the paste which has hardened into particles, and curing the particles in the presence of carbon dioxide to form the capsule.

5 The examples section further hereinbelow demonstrate the method in more detail.

[0071] Embodiments and advantages described for the capsule of the first aspect can be analogously valid for the present method subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples demonstrated hereinbelow, they shall not be iterated for brevity.

10 **[0072]** In various embodiments, the paste comprising the bacterial spore and water, or the paste comprising the bacterial spore, water and the nutrient, may further comprise reactive magnesia cement. In various embodiments, apart from reactive magnesia cement, the paste may comprise waste materials incorporated into the reactive magnesia cement, wherein the waste materials are harmless to the bacteria, whether in spore or
15 vegetative state.

[0073] In various embodiments for forming the paste comprising the bacterial spore, water and the nutrient, the method may comprise mixing the bacterial spore and the water with the reactive magnesia cement first. After the bacterial spore is well dispersed, the nutrient is added. In various embodiments, the nutrient can be added as
20 a dry nutrient powder. Advantageously, when the water together with the bacterial spore are first mixed with the reactive magnesia cement, the reactive magnesia cement particles can first react with water during mixing. Subsequently, when the nutrient (e.g. in dry powder form) is added, the amount of water present is insufficient to render dissolution of the nutrients such that the nutrients remain in a dry powder state and do
25 not react with the bacterial spore (i.e. the nutrient resides in the paste separate from the bacterial spore). If the bacterial spore, water and nutrient are mixed together in the reactive magnesia cement at the same time, potentially, the bacterial spore may contact the nutrients and become activated. Once the bacterial spore is activated, it becomes vulnerable and susceptible to death during the mixing process or addition of other
30 substances. Hence, from such a step of mixing bacterial spore and water together with the nutrient, the incorporation of nutrients inside the same capsule with bacteria may lead to additional bacteria death. Also, some nutrients may be consumed because of the

bacterial spore activation, rendering less nutrients available left for self-healing. That said, in instances where the bacterial spore, water and nutrient are mixed together at the same time, the nutrients are in an aqueous state (i.e. dissolved). In other words, if the bacteria spore is mixed with dry nutrient powder (and/or where water is present in an insufficient amount to render dissolution of the nutrients as mentioned earlier), the bacterial spore advantageously stays inactivated and do not react with the nutrient.

[0074] In various embodiments, curing the particles may comprise curing the particles in the presence of 10 vol% carbon dioxide and 80% relative humidity.

[0075] In various embodiments, the weight ratio of water to the reactive magnesia cement may range from 0.5 to less than 2 (i.e. 0.5:1 to less than 2:1). For example, the weight ratio of water to reactive magnesia cement can be 0.5:1 or less than 2:1. When the weight ratio of water to the reactive magnesia cement exceeds 2, the strength of the capsule may be compromised.

[0076] The present disclosure also provides for a nutrient capsule incorporable into a cement-based material for housing a nutrient which aids a bacterial spore for repairing a crack in the cement-based material. The nutrient capsule comprises an inorganic particle having a surface and a core region, wherein the inorganic particle has a density and a strength which decrease in a direction from the surface to the core region, and wherein the core region houses the nutrient and the capsule is absent of a bacterial spore. The present nutrient capsule differs from the capsule of the first aspect in that the nutrient capsule houses nutrient instead of bacterial spore. As such, understandably, embodiments and advantages described for the capsule of the first aspect can be analogously valid for the present nutrient capsule subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples demonstrated hereinbelow, they shall not be iterated for brevity.

[0077] In various embodiments, the nutrient capsule can be formed of Portland cement or derived from any RMC-based material. The nutrient may reside in the core region, near the surface, and/or at any region between the core region and the surface.

[0078] The present disclosure also provides for a method of forming the nutrient capsule. The method comprises providing a nutrient which aids a bacterial spore for repairing a crack in a cement-based material, forming a paste comprising the nutrient

and water, allowing the paste to harden, converting the paste which has hardened into particles, and curing the particles in the presence of carbon dioxide to form the nutrient capsule.

[0079] Embodiments and advantages described for the capsule of the first aspect and the nutrient capsule can be analogously valid for the method of forming the nutrient capsule subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples demonstrated hereinbelow, they shall not be iterated for brevity. The examples section further hereinbelow demonstrate the method in more detail.

[0080] The present disclosure further provides for a cement-based material incorporated with (i) the capsule described in various embodiments of the first aspect, or (ii) the capsule described in various embodiments of the first aspect and the nutrient capsule.

[0081] Embodiments and advantages described for the capsule of the first aspect can be analogously valid for the present cement-based material subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples demonstrated hereinbelow, they shall not be iterated for brevity.

[0082] In various embodiments, the cement-based material may further comprise a nutrient incorporated in the cement-based material, wherein the nutrient may be incorporated in the cement-based material and external to (i) the capsule described in various embodiments of the first aspect and, when present, (ii) the nutrient capsule. In other words, even if a nutrient capsule is incorporated in the cement-based material, the (additional) nutrient can reside external of the capsule and the nutrient capsule (when present).

[0083] In various embodiments, the cement-based material may be derived from a cement comprising Portland cement.

[0084] In various embodiments, the cement-based material may further comprise a calcium precursor. The calcium precursor may comprise calcium lactate, calcium acetate, calcium glutamate, and/or calcium formate, as non-limiting examples.

[0085] The present disclosure further provides for a method of forming the cement-based material described in various embodiments. The method comprises providing (i)

a capsule described in various embodiments of the first aspect, or (ii) a capsule described in various embodiments of the first aspect and the nutrient capsule, and mixing the capsule, and when present, the nutrient capsule, with a cement to form the cement-based material. Embodiments and advantages described for the capsule of the first aspect and the nutrient capsule can be analogously valid for the present method of forming the cement-based material subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples demonstrated hereinbelow, they shall not be iterated for brevity. The examples section further hereinbelow demonstrate the method in more detail.

5 [0086] In various embodiments, providing (i) the capsule or (ii) the capsule and the nutrient capsule comprises, dispersing the capsule, and when present, the nutrient capsule, homogenously in water to form an aqueous suspension prior to mixing the aqueous suspension with the cement. In certain non-limiting embodiments, providing (i) the capsule or (ii) the capsule and the nutrient capsule, comprises preparing an aqueous solution comprising a nutrient, and mixing the capsule, and when present, the nutrient capsule, into the aqueous solution to form a mixture prior to mixing the mixture with the cement.

[0087] In various embodiments, the method may further comprise mixing a calcium precursor with the capsule, and when present, the nutrient capsule, and the cement. The calcium precursor may include, as non-limiting examples, calcium lactate, calcium acetate, calcium glutamate, and/or calcium formate.

[0088] In various embodiments, the cement may comprise Portland cement.

[0089] The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the present disclosure.

[0090] In the context of various embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

[0091] In the context of various embodiments, the term “about” or “approximately” as applied to a numeric value encompasses the exact value and a reasonable variance.

[0092] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0093] Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

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Examples

[0094] The present disclosure relates to a functionally graded bacteria capsule usable as cement additives to engage in self-healing of concrete and cement-based materials. For brevity, the present functionally graded bacteria capsule may be termed herein a
10 "capsule". The capsule has a gradient density and strength, presenting a gradual reduction of density and strength from the surface to the core region of the capsule.

[0095] Compared to traditional polymer-based and porous capsules which tend to be soft and weak, the present functionally graded capsule is hard and strong. Unlike the addition of traditional polymer-based capsules which leads to reduction of concrete
15 strength, inclusion of the present functionally graded capsule in concrete and cement-based materials improves strength of the concrete and cement-based materials.

[0096] The capsule has better compatibility with the surrounding cement matrix due to the use of inorganic-based material (e.g. RMC – reactive magnesia cement) as the capsule binder.

20 [0097] Compared to traditional carriers which do not provide full protection for bacteria and bacterial spore, the dense surface shell of the present capsules hinders the penetration of alkaline components from the cement matrix and provides better protection for the bacteria. Meanwhile, the porous core with lower alkalinity provides spaces to host bacteria and to ensure long-term viability of bacterial spores.

25 [0098] The present disclosure also relates to a nutrient capsule incorporable into a cement-based material (e.g. concrete) for housing a nutrient which aids a bacterial spore for repairing a crack in the cement-based material.

[0099] The present disclosure also relates to methods of forming aforesaid capsule and nutrient capsules. The present capsule, nutrient capsule, and methods are described in
30 further details, by way of non-limiting examples, as set forth below.

[00100] **Example 1: Introductory Discussion of Present Capsule**

[00101] The present example discusses the present functionally graded bacteria capsule as a cement additive to engage self-healing in concrete and cement-based materials. This capsule has a gradient density and strength from the capsule surface to the core. The dense feature of the surface can provide better protection for the bacteria, while the porous internal environments are favourable for the long-term survival of bacteria and exposure of bacteria when cracks propagate. The application of this capsule is not only limited to the PC-based self-healing concrete. As the developments of alternative binders, the capsule can be applied to any cementitious construction material where encapsulation of bacteria is required (e.g. high alkalinity geopolymer).

5 [00102] To demonstrate the preparation and use of the capsule, the fabrication of capsule using RMC is discussed as a non-limiting example.

[00103] For the preparation of bacteria, the specific bacteria is cultivated using a suitable medium and cultivation conditions. After the bacteria is prepared, centrifugation is required to separate bacteria from spent medium. Afterwards, the supernatant should be removed, and only pellet is left.

15 [00104] For the preparation of the capsule, generally, after the RMC and water used for mixing are measured separately, the water was used to re-suspend the bacteria pellet prepared above. After the bacteria is uniformly dispersed in water, the suspension is poured into RMC. The paste is mixed for about 5 minutes until reaching a homogeneous state. Afterwards, the fresh paste is cast into moulds. There is no restriction on the size of the moulds. After initial hardening for about 2 to 3 days, the hardened pastes are demoulded and crushed into powder using a mortar. Then, the powder is spread on a pan, and subjected to an accelerated carbonation curing with 10% CO₂ and 80% relative humidity for 7 days.

20 [00105] The use of the present capsule in cement-based systems for self-healing is discussed in general as follows. After carbonation curing of the capsules is completed, the capsules are taken out and the weight of capsules is measured according to specific application needs. Afterwards, the ingredients of the mixes are measured accordingly, and the capsules can be either mixed with cement or suspended in water first. If the capsules are mixed with cement, the mixture should be mixed with a mixer at least 1 minute to ensure the capsules are evenly distributed in the cement. If the capsules are mixed with water, the suspension should also be mixed until capsule is well dispersed.

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After the capsules are mixed with cement/water, the rest of the mixing procedure can follow the standard mixing procedure of corresponding cement-based systems. Briefly, for the standard mixing procedure, after the capsules are mixed with cement and/or water, the nutrients are then added to be dissolved therein to form an aqueous mixture.

5 Next, the aqueous mixture was added to cement (if not already present) and mixed in a mixer (e.g. about 5 minutes) until the paste turns homogeneous.

[00106] Example 2: Overview of Method of Forming Capsule and Characterization

[00107] In order to demonstrate the working principle of the present disclosure, the preparation of a RMC-based capsule and its application in Portland cement (PC)-based mixes for self-healing are described as follows.

[00108] In this example, *Bacillus cohnii* was encapsulated in RMC. To prepare the bacteria, vegetative cells were grown aerobically in a sterile growth medium consisting of 5 g/L peptone, 3 g/L meat extract, 4.2 g/L NaHCO₃ and 5.3 g/L Na₂CO₃. After 24 hours of incubation on an orbital shaker (200 rpm) at 25°C, the vegetative cultures were inoculated into a sterile sporulation medium (1% inoculum by volume) for 3-7 days under the same incubation environment. The sporulation medium consisted of 0.2 g/L NH₄Cl, 0.02 g/L KH₂PO₄, 0.225 g/L CaCl₂, 0.2 g/L KCl, 0.2 g/L MgCl₂·6H₂O, 1 ml trace elements solution SL12B (for 1 L sporulation medium), 0.1 g/L yeast extract, 4.2 g/L NaHCO₃, 5.3 g/L Na₂CO₃, and 5.16 g/L sodium citrate. The spores were harvested by centrifugation at 8000-9000 rpm for 10 minutes and the pellets were re-suspended in sterile water. To kill all the vegetative cells, the spore suspension was subjected to pasteurization (20 minutes in a 80°C water bath, followed by 5 minutes in meshed ice). Serial dilutions and spread plate technique were used to determine the colony-forming unit (CFU) concentration of the spore suspension. Afterwards, the suspension was centrifuged at 8000-9000 rpm for 10 minutes. The supernatant was removed, and the pellets were saved for capsule fabrication.

[00109] To fabricate the capsule, RMC, bacteria, and water were measured as shown in table 1 below, respectively. Bacteria was suspended in water and mixed uniformly firstly. Then, the suspension was poured into RMC, and mixed for about 5 minutes until a homogeneous state was reached. The fresh paste was cast into 2.5 cm cube mould and hardened under an ambient condition for 3 days. After demoulding, the cubes were

crushed into powder with a 150 μm mesh and spread on a pan and subjected to an accelerated carbonation curing with 10% CO_2 and 80% relative humidity for 7 days.

[00110] Table 1 – Compositional configuration of RMC-based capsule

RMC (g)	Water (g)	Bacteria (CFU)
40	20	1.02×10^{11}

5 **[00111]** After the capsule was prepared, the composition of the capsule was checked with x-ray diffraction (XRD). The XRD scan was conducted on a Panalytical Xpert Pro using Cu K α radiation (40 kV, 30 mA), with a scanning rate of 0.017° 2 θ /step from 5° to 80° 2 θ .

[00112] The RIR (reference intensity ratio) technique was applied for the quantitative analysis of the phases present by making use of a standard phase with a known proportion, whose integrated intensity is compared to that of the phase of interest. The internal standard used for quantification purposes was fluorite (CaF_2) at 20 wt.%. Calibration curves for magnesia and brucite, which were the components used for quantitative analysis, were straight lines through the origin i.e. $y = kx$, where x is the RIR of the phase analyzed and y is the weight fraction of the component investigated. The value of k was 0.2886 for magnesia and 0.3651 for brucite. The RIR was acquired through dividing the integrated intensity of the strongest line of the phase with that of the standard. Besides, scanning electron microscope (SEM) was adopted to examine the morphology of the capsule. Around 0.1 g of capsules was coated with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. The coated sample was studied with a JEOL JSM-7600 F equipment operating under SEI mode at a voltage of 5.0 kV.

[00113] To investigate the effectiveness of the present capsule for protecting bacteria, bacteria with and without encapsulation were prepared and applied into PC to check the self-healing performance. The amount of capsule, PC, and corresponding nutrients for bacteria were measured according to table 2 below.

[00114] Table 2 – Compositional design of P, PNB and PNC samples for self-healing test

	PC (g)	Water (g)	Calcium lactate (g)	Yeast extract (g)	Bacteria (CFU)	Capsule (g)
P	1500	480	-	-	-	-
PNB	1500	480	30	15	5.95×10^{10}	-
PNC	1500	480	30	15	-	35

[00115] In table 2, P group represents pure PC paste without any additives, while PNB group represents PC paste with bacteria (without encapsulation) and corresponding nutrients. PNC group represents PC paste with the prepared capsule and same nutrients as PNB group. The total bacteria content in the capsule used for PNC group was the same as bacteria used in PNB group. Nutrients were dissolved in water first. After complete dissolution, capsules were added into the solution and mixed until capsules were uniformly distributed in the suspension. Then, the mixture was added into PC and mixed with a mixer for about 5 minutes until homogeneous. The fresh paste was cast into cylinder moulds with diameter of 7.5 cm and height of 2.5 cm. The samples were sealed in an air-tight plastic bag twice to prevent water evaporation. After initial hardening for 1-2 days, the samples were demoulded and cured under the same condition until 28 days.

[00116] At 28 days, the samples were loaded on a servo-hydraulic universal testing machine to generate an initial crack under a loading rate of 0.01mm/s, as shown in FIG. 1A. Afterwards, the initial crack width was measured by Nikon stereoscopic microscope. Then, all the samples were subjected to a wet/dry healing cycle. The wet/dry healing cycle consisted of 1 day submersion in water and 1 day drying under ambient condition. After healing for 5 cycles, the crack closure of the same crack was checked by the microscope.

[00117] To study the influence of RMC-based capsule on the mechanical properties of the matrix, compressive strength tests were conducted for P and PNC samples as indicated in table 2. However, in order to further investigate the single influence of nutrients and capsule on the matrix, another two sets of samples with addition of nutrients and capsules individually were prepared. The compositional configuration of samples casted for compressive strength tests are shown in table 3. The casting and curing method followed the same procedures as described above, except for the size of

mould used for strength test was 50×50×50 mm. At 28 days of curing, the samples were tested under a constant loading rate of 55 kN/min.

[00118] Table 3 – Compositional configuration of samples for compressive strength tests

	PC (g)	Calcium lactate (g)	Yeast extract (g)	Capsules (g)
P	1500	-	-	-
PNC	1500	30	15	35
PN	1500	30	15	-
PC*	1500	-	-	35

5

[00119] FIG. 2 shows the XRD patterns of capsule with introduction of 20% CaF₂. As marked on the pattern, strong peak of fluorite (at 28.2° and 47.0°), unhydrated MgO (at 42.9° and 62.2°) and uncarbonated brucite (at 18.6°, 38.0° and 50.8°) are observed. Besides, minor peaks of hydromagnesite (at 9.6° and 15.2°) and magnesite (at 32.8°) are observed. The existence of magnesite was due to the incomplete calcination during the RMC production process, while the formation of hydromagnesite was due to the accelerated carbonation curing. Follow the RIR calculation, the compositions of the capsule are shown in table 4. As described above, only the surface the RMC-based samples can be carbonated, and the core region of the sample remain brucite. Therefore, around 50% of the capsules was uncarbonated and remained porous brucite, while only around 17% of the capsules presented carbonated phase.

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[00120] Table 4 - Compositions of the RMC-based capsule

Composition	Weight percentage (%)
MgO	30.9
Brucite	51.5
HMCs	17.6

[00121] The morphology of the capsule is shown in FIG. 3. As shown in FIG. 3, the surface of the capsule is fully covered by rosette-like hydromagnesite. The abundant formation of hydromagnesite on the capsule surface together with the existence of large

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amount of brucite in the capsule as indicated by XRD is an evident indication of the density-gradient property of the RMC-based capsule of the present disclosure.

[00122] The self-healing performance of group P, PNB, and PNC are shown in FIG. 4A to 4C, respectively. For each group, crack with width around 200 μm was presented for comparison. FIG. 4A shows that although there was sparse formation of crystals along the edges of the crack on PC samples, the crack was far from completely heal. The crystal formation within the crack was due to further hydration of unreacted cement or carbonation of $\text{Ca}(\text{OH})_2$ in the matrix, which is an intrinsic self-healing property of PC. Similarly, FIG. 4B shows that there was some crystal formation along the crack, but the PNB sample was not able to completely heal. The result indicates the limited self-healing performance when bacteria was added without any protection, which is mainly due to the disability of bacteria survival under such harsh environments. This observation is in line with a study conducted, where bacteria was not able to generate precipitations after embedding in PC for 28 days. Differing from P and PNB group, PNC group (FIG. 4C), where bacteria were encapsulated in the RMC-based capsules, has shown abundant formation of precipitation and led to complete healing of crack under the same curing cycles. The significant crack healing difference between group PNB (bacteria without encapsulation) and PNC (bacteria encapsulated in RMC-based capsule) has indicated the protection efficiency of the presented RMC-based capsule. Besides, since bacteria needs to be exposed so that it can reach nutrients for precipitation, the results have indicated bacteria exposure when crack propagates.

[00123] FIG. 5 shows the compressive strength of P, PNC, PN and PC* samples at 28 days. Comparing pure PC samples with PC+Capsules samples, no significant difference was observed, indicating the addition of capsules did not compromise the strength of the matrix. However, around 17% reduction was caused due to the addition of nutrients by comparing PC samples with PC+Nutrients samples. When capsules and nutrients were applied into PC together (PC+Nutrients+Capsules samples), a minor increase (about 8%) was observed comparing to PC samples. The increase could associate with the pre-consumption of nutrients and pre-precipitation of carbonate phase during the 28-day curing. When fabricating the RMC capsule, there might be some bacteria located on the capsule surface. During mixing and early curing, these bacteria on the surface were exposed to the nutrients and $\text{Ca}(\text{OH})_2$ in the matrix. As a result, the

bacteria spores were able to be activated with the help of yeast extract and subsequently convert calcium lactate (CaL) into calcium carbonate, as shown in equation (1) below. Apart from calcium lactate, other calcium precursors such as calcium acetate, calcium glutamate, and/or calcium formate, can be used. Meanwhile, the CO₂ generated from the reaction can further carbonate Ca(OH)₂ in the matrix.



[00125] Example 3A: General Discussion of a Non-limiting Example of Present Capsule Incorporated with Bacterial Spores

[00126] Examples 3A to 3L demonstrate and discuss the development of a functionally graded reactive magnesia cement-based bacteria spores (RMC-B) capsule for self-healing concrete. The capsule had a gradient structure with a dense shell and a porous and low alkaline core to better protect and to enhance the viability of the bacteria spores. Results showed that the inclusion of the RMC-B capsules did not negatively impact the hydration and the fresh properties of the PC paste and enhanced the 28-day compressive strength of the hardened PC paste by 18%. Specimens with the RMC-B capsules exhibited strong crack healing performance as evidenced by the closure of cracks of few hundred microns and reduction of transport properties due to the dense formation of layered rhombohedral calcite along the entire crack from the surface to the interior.

[00127] Concrete is the most widely used man-made construction material in the world with an annual production of more than 6 billion tons. Due to their limited tensile strength, concrete structures are prone to develop cracks under external loadings. Once the crack forms, it allows the ingress of moisture, carbon dioxide, and aggressive substances to reduce the service life of the infrastructure. Manual repairs are commonly adopted for the remediation of cracks in concrete structures, which is labor-intensive, inconvenient and costly. In the U.S., the estimated annual cost of concrete repair is 18-21 billion dollars. With the goal of eliminating these drawbacks of manual repair, an environmentally friendly bacteria-based technique is described in the present disclosure to realize the self-healing of cracks in concrete structures. The mechanism of this method is to apply dormant bacteria spores (in capsules) with necessary compounds (e.g. nutrients and calcium source) into concrete during mixing. When cracks develop, bacteria spores are exposed and activated upon the ingress of oxygen and water.

Afterwards, the activated bacteria precipitates through its metabolic activities, leading to crack closure.

[00128] Based on this bacteria-based method, studies have been conducted to investigate its feasibility, whereas the disability of bacteria precipitation after being embedded directly (without capsule) into Portland cement (PC)-based mixes for 28 days was reported. The inactivation of bacteria in concrete is generally believed to be associated with the high alkalinity (e.g. pH more than 12) and the densification of the microstructure during hydration. Due to this harsh environment of concrete, encapsulation is advantageous for the protection of bacteria. Traditionally, commonly used encapsulation materials for bacteria in self-healing concrete tend to be polymer-based coating materials (e.g. melamine, hydrogel and epoxy resin) and porous carriers (e.g. expanded clay, biochar, and cellulose fiber). It has been reported that these encapsulations may prolong the life of bacteria in concrete and enhance self-healing but not without significant disadvantages. Firstly, significant strength loss of the matrix has been reported when polymer-based materials were used. For example, the addition of 5% melamine-based microcapsules was shown to lead to 34% decrease in 28-day compressive strength, and 1% addition of modified-alginate hydrogel capsule caused 23% decrease in 28-day compressive strength. The strength reduction was mainly because these polymer-based capsules were often soft materials with much lower strength and stiffness than the PC-based matrix. The inclusion of such capsules created several weak locations in the matrix, therefore reducing the strength. Furthermore, the use of porous carriers did not provide full protection for the bacteria. Generally, after porous carriers were adopted to encapsulate bacteria inside by soaking or vacuum impregnation, no further surface treatment was conducted to seal the openings in the porous carriers. This led to the penetration of the alkaline compounds in PC-based mixes into the porous carriers and contact with bacteria during mixing.

[00129] To overcome these drawbacks and to address concerns regarding mechanical performance, the use of low alkalinity cementitious materials for encapsulation was explored. These materials not only have lower alkalinity which potentially allows the survival of bacteria inside but also have high compatibility with the PC-based matrix. It was reported that using low alkaline sulphoaluminate cement to encapsulate bacteria can enable the self-healing of the crack. At the same time, the 28-day compressive

strength of the matrix was maintained when the dosage of the capsules was 12% by cement mass. In another reported example, use of carbide slag, fly ash, and desulfurized gypsum to prepare a cementitious material to encapsulate bacteria for self-healing was investigated. The results showed that the biocapsule can engage self-healing of cracks
5 of about 500 μm , and the strength of the matrix can be maintained when the dosage of the capsules was below 5% of cement mass. Besides, the study has observed the capsule breakage after cracking, suggesting the high compatibility of the cementitious materials with the PC-based matrix. However, continuous hydration of those cementitious materials resulted in microstructure densification and reduction of porosity of the
10 bacteria capsules. Therefore, the long-term viability of the bacteria inside the capsules was compromised.

[00130] In contrast to reported examples, this present disclosure provides for a graded reactive magnesia cement (RMC)-based bacteria spores capsules for self-healing concrete. RMC, a low alkaline binder (i.e., pH ~ 10.5), was used to encapsulate bacteria
15 spores. Unlike most cementitious materials where the strength gain is through hydration, RMC develops its strength mainly via carbonation which makes the resulting RMC-based bacteria (RMC-B) capsules to possess a unique stable gradient structure with a dense shell and a porous core. The porous core accommodates bacteria spores while the dense shell protects bacteria spores from direct contact with the surrounding
20 cement matrix and provides mechanical strength. The stable microstructure of the RMC-B capsule advantageously promotes long-term viability of bacteria spores.

[00131] In general, bacteria spores capsules were fabricated by encapsulating *Bacillus cohnii* spore in RMC pastes. *Bacillus cohnii* is a non-ureolytic bacteria, which can oxidize calcium lactate to form calcium carbonate. Unlike ureolytic bacteria which lead
25 to ammonia formation during calcium carbonate precipitation, the metabolic precipitating route of *Bacillus congii* does not cause any ammonia burden to the environment. The compositions and morphology of the RMC-B capsules were characterized. Viability of bacteria spores in the RMC capsules was evaluated. Effects of RMC-B capsule addition on hydration and fresh and hardened properties of PC paste
30 were investigated. The capsules were then incorporated together with the nutrients into a PC paste (as a non-limiting example) to evaluate the self-healing performance. The nutrients included calcium lactate (CaL) and yeast extract (YE), where YE helped the

activation of the spores and CaL was the precursor for calcium carbonate precipitation. Apart from calcium lactate, other calcium precursors such as calcium acetate, calcium glutamate, and/or calcium formate, can be used. The self-healing performance was evaluated via observation of crack closure and tests on water passing rate.

5 **[00132] Example 3B: Materials and Methodology - Preparation of Bacteria Spores**

[00133] The vegetative cells of *Bacillus cohnii* (ATCC 51227) were grown aerobically in a sterile growth medium. The vegetative growth medium consisted of 5 g/L peptone, 3 g/L meat extract, 4.2 g/L NaHCO₃ and 5.3 g/L Na₂CO₃. After 1 days of incubation
10 on an orbital shaker (200 rpm) at 25°C, the vegetative cultures were inoculated into a sterile sporulation medium (1% inoculum by volume) for 5 days under the same incubation environment. The sporulation medium consisted of 0.2 g/L NH₄Cl, 0.02 g/L KH₂PO₄, 0.225 g/L CaCl₂, 0.2 g/L KCl, 0.2 g/L MgCl₂·6H₂O, 1 ml trace elements solution SL12B (for 1L sporulation medium), 0.1 g/L yeast extract, 4.2 g/L NaHCO₃,
15 5.3 g/L Na₂CO₃, and 5.16 g/L sodium citrate. Afterwards, the spores were centrifugated at 8000 rpm for 10 minutes. The supernatant was removed, the pellet was left and re-suspended in sterilized water. The centrifugation and re-suspension were repeated twice to wash the bacteria cells and keep the cells free from spent medium. To kill all the vegetative cells, the spore suspension was subjected to pasteurization (20 minutes in a
20 80°C water bath, followed by 5 minutes in mashed ice). Serial dilutions and spread plate technique were used to determine the colony-forming unit (CFU) concentration of the spore suspension. The spores were then stored in a 4°C fridge before use.

[00134] Example 3C: Materials and Methodology - Fabrication of Present RMC-Based Bacteria Spores Capsule (RMC-B Capsule)

25 [00135] To fabricate the functionally graded RMC-based bacteria spores capsule (RMC-B capsule), the spore pellets (1.02×10^{11} CFU) were first mixed with 20 g water, forming a well-dispersed suspension solution. The spore suspension was then added into 40 g RMC powder and mixed with a hand mixer for around 5 minutes until a homogeneous state was reached. The RMC used in this non-limiting example, whose
30 chemical composition is shown in table 5 below, was purchased from Richard Baker Harrison Ltd. (UK). Its reactivity, measured by the time required for the neutralization of 0.25 M of acetic acid by 5 g of RMC, was recorded as 520 s.

[00136] Table 5 - Chemical composition of RMC

Composition	Weight percentage (%)
MgO	> 91.5
SiO ₂	2.0
CaO	1.6
R ₂ O ₃	1.0
LOI	4.0

[00137] The fresh pastes were then cast into 2.5 cm cube molds and hardened for 3 days under ambient conditions. After demoulding, the cubes were crushed into powders using a mortar, and sieved through a 150 μm mesh. The powders were spread onto a pan and subjected to accelerated carbonation curing under 30°C with 10% CO₂ and 80% relative humidity (RH) for 7 days. After carbonation was completed, the particle size distribution of the capsules was investigated by a laser diffraction particle size analyzer (Beckman Coulter LS 13 320) and the results are shown in FIG. 6.

[00138] Around 0.1 g capsules were freeze-dried for 1 day, and then coated with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. The coated capsules were examined by scanning electron microscopy (SEM, JEOL JSM-7600 F) under the secondary electrons imaging (SEI) mode at a voltage of 5.0 kV.

[00139] To quantify the crystallographic structure of the capsules, XRD and the RIR method were adopted. The carbonated capsules were further crushed and sieved with a 75 μm mesh. 20 wt.% fluorite (CaF₂) was introduced to the crushed and sieved capsules as the internal standard. The mixture was freeze dried for 1 day, and then scanned by Panalytical Xpert Pro using Cu K α radiation (40 kV, 30 mA), with a scanning rate of 0.017° 2 θ /step from 5° to 80° 2 θ . In the RIR calculations, calibration curves for magnesite and brucite, which were the components used for quantitative analysis, were straight lines through the origin i.e. $y = kx$, where x is the RIR of the phase analyzed and y is the weight fraction of the component investigated. The value of k was 0.2886 for magnesite and 0.3651 for brucite. The RIR was acquired through dividing the integrated intensity of the strongest line of the phase with that of the standard. For the calculation of HMC phases in the capsules, the amount allocated to magnesite originally

present in the raw material (RMC) was deducted from the rest. To obtain the magnesite content in RMC, thermogravimetric analysis (TGA) was conducted on a Perkin Elmer TGA 4000 equipment from 50 to 900°C, with a heating rate of 10 °C/min under nitrogen flow.

- 5 [00140] The viability of the bacteria spores in the RMC-B capsules was quantified according to a method as follows. Firstly, the RMC-B capsules were crushed with a mortar and sieved with a 53 µm mesh. 1 g of the sieved powder was then suspended in 10 ml distilled water and vortexed for around 1 minute. Afterwards, the suspension was treated in an ultrasonic water bath (70 W, 42 kHz) for 20 minutes. The supernatant was
- 10 then taken out for serial dilution and spread plate counting. The followed procedure is shown FIG. 7. Three replicates were prepared for each test. The viability of bacteria spores in the PC capsules was also evaluated as the control. 1.15×10^9 CFU bacteria spores were added in a PC paste (50 g cement with 16 g of water) followed the same mixing procedure as the RMC-B capsules and crushed into powder. The same
- 15 quantification procedure was carried out to investigate the viability of bacteria spores in the PC-B capsules at the age of 1, 8, 14 and 28 days.

[00141] Example 3D: Materials and Methodology - Effects of RMC-B Capsule Addition on Hydration and Fresh and Hardened Properties of PC Paste

- 20 [00142] RMC-B capsules were incorporated into a PC paste to study their effects on heat of hydration, flowability, initial setting time, and compressive strength of the PC paste. Table 6 shows the compositional configuration of the PC paste with the inclusion of RMC-B capsules. A control composition with the same compositional configuration but without the inclusion of RMC-B capsule was also prepared.

- 25 [00143] Table 6 – Compositional configuration to study effects of RMC-B capsule addition on hydration and fresh and hardened properties of PC paste

Compositional Sample	PC (g)	Water (g)	RMC-B capsule (g)
P-C	1500	480	35
P	1500	480	-

[00144] To prepare the P-C mix, the RMC-B capsules were first mixed in water. The suspension was then added into PC and mixed in a mixer for ~5 minutes until homogeneous. The heat of hydration was measured by means of the isothermal

calorimetry test at 30°C in an I-Cal 8000 HPC calorimeter (Calmetrix) in accordance with ASTM C1679. The flowability of the paste was determined in accordance with ASTM C1437 using a flow table (63-L0040/A, Controls). The initial setting time was measured in accordance with ASTM 191 using an automatic Vicat apparatus (EN 196-3, Testing Bluhm & Feuerherdt GmbH).

[00145] To determine the compressive strength, fresh pastes were cast into 50 mm cubic molds, sealed in an air-tight plastic to prevent water evaporation, and placed in ambient conditions (25°C, 80% RH). The cubic specimens were demolded after 2 days and cured under the same conditions for another 26 days. Afterwards, the compressive strength of the specimens was tested at the age of 7, 14 and 28 days on a compression machine (ToniTechnik Baustoffprüfsysteme) operated at a loading rate of 55 kN/min.

[00146] Example 3E: Materials and Methodology - Self-Healing Properties of Capsules

[00147] The fabricated RMC-B capsules were used to prepare specimens (sample PNC) to evaluate the self-healing performance as shown in table 7 below. Two control compositions were also prepared: (i) sample P, a PC paste without the inclusion of RMC-B capsules and the nutrient, was to reveal crack healing (if any) due to further hydration or carbonation of the PC matrix; and (ii) sample PNS was designed to study the self-healing performance of PC paste with the inclusion of unprotected bacteria spores at the same concentration (CFU) level.

[00148] Table 7 – Compositional configuration of specimens prepared for self-healing tests

Sample Name	PC (g)	Water (g)	CaL (g)	YE (g)	RMC-B capsule (g)	Bacteria spores (CFU)
PNC	1500	480	30	15	35	-
PNS	1500	480	30	15	-	5.95×10^{10}
P	1500	480	-	-	-	-

[00149] PC used in this example was purchased from EnGro Ltd (Singapore), complying with SS EN 197-1: 2014. The chemical composition of PC is shown in table 8 below. The calcium lactate and yeast extract were purchased from Sigma-Aldrich.

Apart from calcium lactate, other calcium precursors such as calcium acetate, calcium glutamate, and/or calcium formate, can be used.

[00150] Table 8 - Chemical compositions of the PC

Composition	Weight percentage (%)
SiO ₂	20-25
Al ₂ O ₃	5-6
Fe ₂ O ₃	2-3
CaO	60-65
MgO	1-2
Na ₂ O	0.2-0.3
K ₂ O	0.4-0.5
C ₃ A	5-10
Free lime	0.5-1.0
LOI	2-3

- 5 **[00151]** To prepare the composition, the nutrients (CaL and YE) were first mixed with water until they were fully dissolved. RMC-based capsules (or bacteria spores) were then added into the solution and mixed until they were well dispersed. The suspension was added into the dry PC powder and mixed for about 5 minutes until homogeneous. The fresh paste was cast into cylinder molds (height of 2.5 cm, diameter of 7.5 cm),
 10 initially filling half of the mold. A circular steel mesh (diameter of 7 cm) was then placed inside, followed by covering the steel mesh with the fresh paste and filling the mold until full as shown in FIG. 8A and 8B. The specimens were sealed in an air-tight plastic to prevent water evaporation. The specimens were demolded after 2 days and cured in the same condition for another 26 days.
- 15 **[00152]** Pre-cracking of the cylinder specimens at 28 days was carried out by means of splitting tension via a servo-hydraulic universal testing machine (MTS Landmark 250 kN), operated at a constant rate of 0.01 mm/s, as shown in FIG. 1A. After pre-cracking and unloading, a split crack was form in the center of the specimen. The crack width was measured at five marked locations along the crack (FIG. 1B) by a stereoscopic
 20 microscope (Nikon SMZ 745T).

[00153] Furthermore, the water passing rate of the pre-cracked specimens was determined by a constant water head method. As shown in FIG. 9, the constant water head was created by a PVC pipe with a height of 10 cm and diameter of 6 cm. The pipe was attached to the top surface of the specimen and sealed with waterproof plasticine.

5 A constant water head of 10 cm was maintained for a duration of 5 minutes to allow water to pass through the pre-cracked specimens. The amount of water collected in the bowl below was measured and the water passing rate was calculated as shown in equation (2) below, where V is the volume of the water passing through the specimen; L is the specimen thickness in the direction of flow; A is the cross-sectional area subject to flow; h is the constant water head; and t is the duration of flow. For each specimen, 10 the test was carried out 3 times, and the mean and standard deviation values were reported.

$$[00154] \text{ Water passing rate} = \frac{VL}{Aht} \quad (2)$$

[00155] Three specimens were prepared for each group, and the specimens were then 15 subjected to wet/dry conditioning regimes, during which they were fully submerged in water (25°C) for 24 hours, followed by drying in the ambient air (25°C, 80% RH) for another 24 hours. Specimens of different mix designs were not placed in the same containers for wet/dry conditioning to avoid cross-contamination between different mixes. Conditioning water was replaced and replenished after each cycle. Crack widths 20 at the five marked locations and the water passing rate after five wet/dry conditioning cycles were determined. The crack closure (%) and water passing rate reduction (%) were calculated in accordance with equations (3) and (4), respectively.

$$[00156] \text{ Crack closure (\%)} = \frac{\text{Crack width (pre-cracking)} - \text{crack width after wet/dry cycles}}{\text{Crack width (pre-cracking)}} \quad (3)$$

$$[00157] \text{ Water passing rate reduction (\%)} =$$

$$25 \quad [00158] \frac{\text{Water passing rate (pre-cracking)} - \text{water passing rate after wet/dry cycles}}{\text{Water passing rate (pre-cracking)}} \quad (4)$$

[00159] Afterwards, small samples were cut from the specimens of Mix PNC by means of a precision saw (Buehler IsoMet 1000), as shown in FIG. 10A to 10C. Samples were extracted from the cracked location to observe healing products (if any) on the specimen surface (FIG. 10A). To check the internal healing products, specimens were fractured 30 to expose the interior of the healed crack (FIG. 6B). Samples were extracted from the uncracked location to observe any precipitation on the specimen surface (FIG. 6C). The

samples were freeze-dried for 3 days to completely remove internal moisture, followed by coating with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. All samples were examined by SEM (JEOL JSM-7600 F) under the SEI mode at a voltage of 5.0 kV.

5 **[00160] Example 3F: Results and Discussion – RMC-B Capsules**

[00161] The SEM images of the fabricated RMC-B capsules are shown in FIG. 11. The surface morphology of the capsules composed of disk-like crystals, which resembled the morphology of hydromagnesite.

[00162] The XRD pattern of the capsules is shown in FIG. 2. Intense fluorite peaks were observed at 28.3° and 47.0° 2θ due to the addition of 20% standard phase. Major peaks of MgO (at 42.9° and 62.3° 2θ) and brucite (at 18.6° and 38.0° 2θ) were also recorded, indicating the presence of uncarbonated brucite even after accelerated carbonation curing. Minor peaks of hydromagnesite and magnesite were found at 15.2° and 32.8° 2θ, respectively. The existence of magnesite was attributed to the incomplete calcination of the parent material during the production of RMC, while hydromagnesite was the main carbonation product that formed during accelerated carbonation curing.

[00163] To calculate the content of carbonate phase (hydromagnesite) in RMC-B capsules, the amount of magnesite (MgCO₃) needed to be deducted. Accordingly, the percentage of magnesite in the raw material (RMC) was calculated based on the weight loss of RMC between 500-900°C, as shown in FIG. 12. The decomposed phases at different temperatures and the corresponding weight loss are marked accordingly in the figure. From 50°C to 300°C, the main weight loss was due to the loss of free water in RMC, followed by the weight loss caused by the decomposition of brucite from 300°C to 500°C. From 500°C to 900°C, the loss was related to the decarbonation of magnesite in the RMC, as shown in equation (5).



[00165]
$$W_{\text{Ms}} = \frac{M_w(\text{Ms})}{M_w(\text{CO}_2)} \times 2.61\% \quad (6)$$

[00166] The weight loss associated with decarbonation was 2.61%, corresponding to 5% magnesite content in RMC, which was calculated by using equation (6), where W_{Ms} is the weight fraction of magnesite, $M_w(\text{Ms})$ is the molecular weight of magnesite and $M_w(\text{CO}_2)$ is the molecular weight of CO₂. The composition of the capsule calculated

by XRD analysis is shown in table 9 below. The RMC-B capsules consisted of around 31% unhydrated MgO, 51% brucite and 13% HMC phases.

[00167] Table 9 – Composition of the RMC-B capsules

Composition	Weight fraction (%)
MgO	30.9
Mg(OH) ₂	51.5
Magnesite	5.0
HMCs	12.6

5 **[00168]** From the surface morphology observations and phase composition results, the structure of the RMC-B capsule is shown schematically in FIG. 13. As can be seen, the resulting RMC-B capsule had a gradient structure with a dense HMCs shell (12.6%) and a porous core (52% brucite and 31% unreacted MgO). This was because MgO
 10 hydrated and formed brucite first. After carbonation, brucite near the capsule surface transformed to a range of HMCs with dense microstructure and the core consisted of mainly brucite and unhydrated RMC with high porosity. The dense HMCs formed on the capsule surface (i.e., shell) hindered further penetration of CO₂ towards the capsule core. As a result, the RMC-B capsules shall have a stable microstructure (i.e., a hard shell and soft core) unlike other cement-based capsules where hydration continuous to
 15 refine the internal microstructure. The porous core accommodated bacteria spores while the dense shell protected bacteria spores from direct contact with the surrounding cement matrix and provided mechanical strength. The stable microstructure of the resulting RMC-B capsule shall promote long-term viability of bacteria spores.

20 **[00169] Example 3G: Results and Discussion - Viability of Bacteria Spores in RMC-B Capsule**

[00170] FIG. 14 has plotted the number of viable bacteria spores in the PC-B capsule. As can be seen, the number of viable bacteria spores reduced dramatically by four orders after 1 day in the PC matrix. The number of viable bacteria spores continued to reduce with the age of PC until lower than the detection limit of the adopted method at
 25 the age of 28 days. This highlighted the vulnerability of bacteria spores in PC-based matrix due to the harsh environment of PC.

[00171] FIG. 15 shows the number of viable bacteria in the RMC-B capsule. As can be seen, the viability of the bacteria spores in the RMC matrix was much higher than that in the PC matrix. This may be attributed to the low alkalinity of the RMC system which was reported to have an internal pH of around 9.9-10.5, providing a more friendly environment for the survival of bacteria spores. Furthermore, the porous core of the RMC-B capsule may preferably provide space to accommodate bacteria spores.

[00172] **Example 3H: Results and Discussion - Effects of RMC-B capsule addition on hydration and fresh and hardened properties of PC paste**

[00173] FIG 16 shows the evolution of hydration heat and the corresponding cumulative heat during the first 72 hours for the PC paste with (sample P-C) and without (sample P) the inclusion of RMC-B capsules. As can be seen, peak of hydration of both mixes took place at around the 9th hour, indicating that the addition of RMC-B capsules did not alter the hydration rate of PC paste. Furthermore, both mixes showed comparable accumulated heat after 72 hours of hydration, suggesting the minimal effect of the capsules on the hydration process at early ages.

[00174] Table 10 below summarizes the flowability and initial setting time of the P-C and P mixes. As can be seen, the control group (sample P) had a flowability of 69% and an initial setting time of 187 min. The inclusion of RMC-B capsules (Mix P-C) did not introduce significant impacts on flowability and initial setting time of the PC paste. This may be attributed to the formation of HMCs (mainly hydromagnesite) shell, which is chemically stable in the alkaline environments such as PC paste, of the functionally graded RMC-B capsule via carbonation. As a result, the inclusion of RMC-B capsules did not introduce significant impacts on the hydration and fresh properties of the PC paste.

[00175] Table 10 - Flowability and initial setting time of the Mix P and Mix P-C

Sample Name	Flowability (%)	Initial setting (min)
P-C	67	209
P	69	187

[00176] FIG. 17 compares the compressive strength of the two mixes at the age of 7, 14 and 28 days. As can be seen, PC paste with the inclusion of RMC-B capsules (sample P-C) showed higher compressive strength than the control mix (sample P). This was

particularly pronounced at early ages. The 7-day strength of sample P-C (63.4 MPa) was 26% higher than that of sample P (50.3 MPa). At the age of 14 days, sample P-C (69.5 MPa) was 21% stronger than sample P (57.6 MPa). When it reached 28 days, the strength of sample P-C (74.1 MPa) was still higher than Mix P (62.8 MPa) by 18%.

5 This indicated the RMC-B capsules were not the strength limiting phase in the hardened PC paste unlike the typical soft self-healing capsules. Furthermore, this suggested the resulting functionally graded RMC-B capsules were strong and highly compatible to the PC matrix perhaps due to the formation of hard HMCs shell via carbonation. Further study is necessary to better understand the interface transition zone between the RMC-
10 B capsule and the surrounding PC matrix to reveal the strength enhancement mechanisms.

[00177] Overall, these results suggested that the addition of the RMC-B capsules did not alter the heat of hydration, flowability, and initial setting time of PC paste. Furthermore, the incorporation of the capsules increased the compressive strength of
15 the matrix. This suggested the newly developed functionally graded RMC-B capsules with hard HMCs shell were chemically stable and compatible with the surrounding PC matrix.

[00178] **Example 3I: Self-Healing Performance – Surface Crack Closure**

[00179] Crack width after 5 wet/dry conditioning cycles was plotted against the original crack width, as shown in FIG. 18. For each mix, 3 specimens were measured,
20 with 5 observation points on each specimen. Thus, overall, there are 15 data points in each plot. The 45° black solid line represents no crack closure, whereas the grey dashed line indicates 50% crack closure. As can be seen, all cracks in the sample PNC reduced their width significantly after 5 wet/dry conditioning cycles while minimum crack
25 closure rate were found in samples PNS and P. Table 11 calculates the average crack closure rate of each mix. As can be seen, the three mixes had comparable crack width of around 220-250 μm before the wet/dry conditioning. After 5 cycles of wet/dry conditioning, both samples PNS and Mix P revealed marginal crack closure rates of about 20% while PNC achieved a significant crack closure rate of around 92%. This
30 highlighted the effectiveness of the RMC-B capsules to engage self-healing in PC paste. The marginal crack closure in PNS and P specimens was likely due to further hydration

and carbonation as the mix had a low water-to-cement ratio of 0.32 and thus plenty unhydrated cement can be expected at the age of 28 days.

[00180] Table 11 - Average crack closure rate after 5 wet/dry conditioning cycles

Sample Name	Original crack width (μm)	Crack width after 5 wet/dry cycles (μm)	Average crack closure rate (%)
PNC	243 $\pm$ 28	19 $\pm$ 31	92.8 $\pm$ 11.0
PNS	227 $\pm$ 52	180 $\pm$ 49	20.1 $\pm$ 16.2
P	240 $\pm$ 19	192 $\pm$ 30	20.2 $\pm$ 8.3

- 5 **[00181]** Representative optical microscope images of a crack in each mix before and after 5 cycles of wet/dry conditioning are presented in FIG. 19A to 19C. Sample PNC (FIG. 19A) revealed a notable healing when compared to samples PNS (FIG. 19B) and P (FIG. 19C). Accordingly, sample PNC achieved a complete crack closure enabled by the abundant formation of healing products within the crack, whereas samples PNS and
- 10 P showed sparse formation of white crystals along the edge of the crack. It was worth noting that the formation of healing products was not only limited to within the crack, but also on the entire surface of the PNC specimen after the wet/dry conditioning cycles. This indicated the high bioactivity of the RMC-B capsules in the PC paste.

[00182] Example 3J: Self-Healing Performance – Water Passing Rate

- 15 **[00183]** FIG. 20 compares the average water passing rate reduction of the 3 mixes. Overall, the results were in agreement with the crack closure rate results. The water passing rate reduction of samples PNS and Mix P were below 25% while that of the PNC was more than 80%. The resistance of the healed crack to the water passing is a key parameter to evaluate the healing performance of the crack as the crack can present
- 20 a weak point in the matrix, enabling the ingress of water and aggressive substances. The resistance is directly associated with the crack closure performance and the density of the crystal formation within the crack. The high resistance of the healed sample PNC to the water passing has indicated the dense nature of the healing products and the overall high healing efficiency of the RMC-B capsules in the PC paste.

- 25 **[00184] Example 3K: Self-Healing Performance – Microstructure of Healing Products**

[00185] FIG. 21A to 21C show the morphology and microstructure of healing products from different location of the Mix PNC specimen. As can be seen, all 3 figures revealed the formation of layered rhombohedral calcite, which was in line with the findings of previous studies using the same type of bacteria and nutrient. The healing products not only formed on the crack surface, but also along the crack depth direction. The dense formation of calcite along the entire crack from the surface to the interior led to the significant reduction of water passing rate in the PNC specimen. The abundant formation of healing products was not just within the crack, but also on the specimen surface as shown in FIG. 21C, where the dense formation of calcite crystals and the presence of gel-like C-S-H from further hydration were observed.

[00186] **Example 3L: Summary for Examples 3A to 3K**

[00187] Examples 3A to 3K demonstrate for the development of the present graded RMC-B capsule for self-healing concrete. The capsule had a gradient structure with a dense HMCs shell and a porous and low alkaline core to better protect and to enhance the viability of the bacteria spores. Results showed that the inclusion of the RMC-B capsules did not negatively impact the hydration, the initial setting time, and the flowability of the PC paste. Furthermore, the 28-day compressive strength of the hardened PC paste incorporating the RMC-B capsules was 18% stronger than that of the control. This suggested the capsules were strong and highly compatible to the PC matrix. The developed functionally graded RMC-B capsules with hard HMCs shell were chemically stable and compatible with the surrounding PC matrix.

[00188] Specimens with the RMC-B capsules exhibited strong crack healing performance as evidenced by the high crack closure rate of 93% and high water passing rate reduction rate of 80% for wide crack of few hundred microns after only five wet/dry conditioning cycles. Microstructure observations revealed the dense formation of layered rhombohedral calcite along the entire crack from the surface to the interior. These highlighted the effectiveness of the present graded RMC-B capsules for self-healing concrete.

[00189] **Example 4A: A Non-limiting Example of RMC-B-N Capsule**

[00190] Examples 4A and 4B relate to a RMC-B-N capsule, which is a self-contained system with bacteria and nutrients encapsulated in RMC together.

[00191] Bacteria spores of *Bacillus cohnii* was prepared followed same procedure as described in the RMC-B capsule system. Afterwards, to fabricate the RMC-B-N capsule, RMC, water, bacteria and nutrients were measured and prepared according to table 12. Bacteria was suspended in water and mixed uniformly firstly. Then, the suspension was poured into RMC, and mixed for around 5 minutes until a homogeneous state was reached. This allows the RMC to first react with the water. Then the pre-mixed yeast extract (YE) and calcium lactate (CaL) powder was added into the paste slowly. As the RMC has earlier reacted with water, the amount of water present when the nutrient (e.g. the yeast extract) is added is insufficient to dissolve the nutrient. Apart from calcium lactate, other calcium precursors such as calcium acetate, calcium glutamate, and/or calcium formate, can be used. The mixture was mixed in the mixer for another 5 minutes until homogeneous again. The fresh paste was cast into 2.5 cm cube mould and hardened under an ambient condition for 3 days. After demoulding, the cubes were crushed into small chips and subjected to an accelerated carbonation curing with 10% CO₂ and 80% relative humidity for 1 day. Afterwards, the chips were taken out and further grinded with a mortar and sieved with a 150 µm mesh. The sieved particles were then spread on a pan and subjected to the same accelerated carbonation curing condition for another 6 days.

[00192] Table 12 – Compositional configuration of RMC-based capsule

RMC (g)	Water (g)	Bacteria (CFU)	YE (g)	CaL (g)
23.3	11.7	5.95×10^{10}	15	30

20

[00193] After capsule was prepared, the composition of capsule was checked with XRD. The XRD scan was conducted on a Panalytical Xpert Pro using Cu K α radiation (40 kV, 30 mA), with a scanning rate of 0.017° 2 θ /step from 5° to 80° 2 θ .

[00194] The RIR technique was applied for the quantitative analysis of the phases present by making use of a standard phase with a known proportion, whose integrated intensity is compared to that of the phase of interest. The internal standard used for quantification purposes was fluorite (CaF₂) at 20 wt.%. Calibration curves for magnesia and brucite, which were the components used for quantitative analysis, were straight lines through the origin i.e., $y = kx$, where x is the RIR of the phase analyzed and y is the weight fraction of the component investigated. The value of k was 0.2886 for

30

magnesia and 0.3651 for brucite. The RIR was acquired through dividing the integrated intensity of the strongest line of the phase with that of the standard. Besides, scanning electron microscope (SEM) was adopted to examine the morphology of the capsule. Around 0.1 g of capsules was coated with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. The coated sample was studied with a JEOL JSM-7600 F equipment operating under SEI mode at a voltage of 5.0 kV.

[00195] To investigate the self-healing performance of the RMC-B-N capsule, P-BNC group as was designed with the incorporation of the capsules in the PC paste. As a comparison, pure PC paste without capsules addition was designed, and the specific mix designs of the two groups are shown in table 13. When preparing P-BNC group, the prepared capsules were measured and mixed with PC in a mixer first for 3~5 minutes until the capsules were well dispersed in the cement. Afterwards, the prepared water was mixed with the powder in the mixer for about 5 minutes until homogeneous. As for group P, the water was directly mixed with the cement for around 5 minutes until homogeneous. The fresh pastes were cast into cylinder molds (height of 2.5 cm, diameter of 7.5 cm), initially filling half of the mold. A circular steel mesh (diameter of 7 cm) was then placed inside, followed by covering the steel mesh with the fresh paste and filling the mold until full. The specimens were sealed in an air-tight plastic to prevent water evaporation. The specimens were demolded after 2 days and cured in the same condition for another 26 days.

[00196] Table 13 – Compositional configuration of specimens prepared for self-healing tests

Sample Name	PC (g)	Water (g)	RMC-B-N capsule (g)
P-BNC	1500	480	80
P	1500	480	-

[00197] At the age of 28 days, the specimens were pre-cracked followed the same procedure as described in the pre-cracking of RMC-B capsule system. The initial crack width and water passing rate were measured and calculated followed the same methods demonstrated in the RMC-B capsule system. After the initial measurement, the specimens were subjected to wet/dry conditioning cycles in water. Specifically, one conditioning cycle included 24 hours full submersion in water (25°C) and followed by

another 24 hours of drying in the ambient air (25°C, 80% RH). After every 10 cycles of conditioning, the crack width and water passing rate were measured and calculated again. The crack closure (%) and water passing rate reduction (%) were calculated in accordance with equations (7) and (8), respectively.

$$5 \quad [00198] \text{ Crack closure (\%)} = \frac{\text{Crack width (pre-cracking)} - \text{crack width after wet/dry cycles}}{\text{Crack width (pre-cracking)}} \quad (7)$$

$$[00199] \text{ Water passing rate reduction (\%)} =$$

$$[00200] \frac{\text{Water passing rate (pre-cracking)} - \text{water passing rate after wet/dry cycles}}{\text{Water passing rate (pre-cracking)}} \quad (8)$$

[00201] To examine the influence of the RMC-B-N capsules on the mechanical property of the matrix, the compressive strength of group P-BNC and P were measured.

10 The mixing and preparation of the pastes followed the same procedure as above described. Afterwards, the fresh pastes were cast into 50 mm cubic molds, sealed in an air-tight plastic to prevent water evaporation, and placed in ambient conditions (25°C, 80% RH). The cubic specimens were demolded after 2 days and cured under the same conditions for another 26 days. Afterwards, the compressive strength of the specimens
15 was tested at the age of 7, 14 and 28 days on a compression machine (ToniTechnik Baustoffprüfsysteme) operated at a loading rate of 55 kN/min.

[00202] **Example 4B: Results and Discussion of RMC-B-N Capsule of Example 4A**

[00203] The SEM image of the fabricated RMC-B-N capsules are shown in FIG. 22. The surface morphology of the capsules composed of needle-like crystals, which
20 resembled the morphology of nesquehonite.

[00204] The XRD pattern of the resulted RMC-B-N capsules is shown in FIG. 23. Intense fluorite peaks were observed at 28.3° and 47.0° 2θ due to the addition of 20% standard phase. Peaks of MgO at 42.9° and brucite at 62.3° 2θ were also recorded, indicating the presence of uncarbonated brucite even after accelerated carbonation
25 curing. Distinct peaks of nesquehonite were observed at 23.1° and 29.5°, and magnesite was found at 32.6° 2θ, respectively. The existence of magnesite was attributed to the incomplete calcination of the parent material during the production of RMC, while nesquehonite was the main carbonation product that formed during accelerated carbonation curing.

30 [00205] The composition of the RMC-B-N capsule calculated by XRD analysis is shown in table 14 below. The RMC-B-N capsules consisted of around 11.9%

unhydrated MgO, 7.7% brucite, 9.5% HMC phases and 65.9% nutrients. The morphology and the composition of the capsules revealed that the surface of the capsules were dense HMCs, while the core region was mainly composed of porous brucite, MgO and nutrients. Essentially, the resulted RMC-B-N capsule was also a functionally graded capsule system.

[00206] Table 14 - Composition of the RMC-B-N capsules

Composition	Weight fraction (%)
MgO	11.9
Mg(OH) ₂	7.7
Magnesite	5.0
HMCs	9.5
Nutrients (YE and CaL)	65.9

[00207] Crack width after 10, 20 and 30 wet/dry conditioning cycles was plotted against the original crack width, as shown in FIG. 24A to 24C. For each compositional configuration, 3 specimens were measured, with 5 observation points on each specimen. Thus, overall, there are 15 data points in each plot. The 45° black solid line represents no crack closure, whereas the grey dashed line indicates 50% crack closure. It can be seen from each figure that, after subjected to wet/dry conditioning cycles, both group P-BNC and P showed crack healing effect as the crack width reduced comparing to original crack width before conditioning. Nevertheless, the healing efficiency of the two groups were significantly different. FIG. 24A clearly shows that after 10 cycles of healing, all the cracks in group P fell between the 2 lines which represented crack closure rates were less than 50%. Differently, in group P-BNC, it can be seen that majority of the cracks, except for a few cracks larger than 300 μm, fell below the grey dash line which represented crack closure rate over 50%. Meanwhile, some cracks between 200 μm ~ 300 μm almost fell on the x-axis, which represented 100% of crack closure. As the condition cycles proceeded to 20 cycles and 30 cycles, the cracks in group P remained near the grey line (i.e., 50% crack closure). Differently, the cracks in group P-BNC kept reducing, and at 30 cycles, majority of the cracks were near the x-axis. Specifically, the cracks between 200μm ~ 300μm had robust healing as nearly all the cracks in this range had 100% of crack closure. The photos of a crack in group P-

BNC at different conditioning cycles were shown FIG. 25A to 25C to demonstrate the healing process.

[00208] The average water passing rate reduction of group P-BNC and group P are shown in FIG. 26. Over the whole conditioning process, both groups showed transport property recovery, which was associate with the crack healing. When comparing the two groups, it was clear that the water passing rate reduction of P-BNC was significantly higher than P regardless of conditioning cycles. At early age of conditioning (i.e., 10 cycles), the reduction of group P-BNC already reached near 60%, while group P was near 10%. Finally at 30 cycles, the water passing rate reduction of group P was around 23.8%, while that of group P-BNC was around 73.8% which was more than 3 times than the control group. Overall, the crack closure and water passing rate test results have revealed the effectiveness of the RMC-B-N capsules regarding crack repairing and transport property recovery.

[00209] To study the influence of the RMC-B-N capsules on the strength of the PC paste, compressive strength of PC pastes with and without the addition of the capsules were tested and shown in FIG. 27, respectively. Generally, the addition of the capsule did not compromise the strength of the matrix over the whole curing period. At 28 days, the incorporation of the RMC-B-N capsules even increased the strength by 8.7%, as the group P-BNC had strength of 68.75 MPa and group P was 62.76 MPa.

[00210] **Example 5: General Discussion of a Non-limiting Example of RMC-N and RMC-B Capsule System (Dual Capsule System)**

[00211] This example demonstrates for RMC-N and RMC-B dual capsule system. RMC-N capsule only contains nutrients in the carbonated RMC capsule, while RMC-B is the capsule developed where only bacteria was encapsulated in the carbonated RMC capsule. RMC-B capsule is already described in examples 1 to 3L. When used as self-healing additives, both RMC-B capsule and RMC-N capsules should be incorporated into PC (dual capsule system).

[00212] For such a dual capsule system, instead of using carbonated RMC, certain amount of PC from the matrix can be used to encapsulate nutrients since the encapsulation of nutrients has no requirement on the alkalinity of the encapsulation materials. Besides, PC usually has higher strength than RMC, so potentially using PC to fabricate the nutrient capsule can bring less mechanical loss or do not cause any

mechanical loss to the matrix. Three different compositional configurations of the PC-based nutrients capsule were prepared, as shown in table 15 below.

[00213] Table 15 – Composition configuration of the PC-based capsule

Sample Name	PC (g)	Water (g)	YE (g)	CaL (g)
PC-N-80	80	25.6	15	30
PC-N-40	40	12.8	15	30
PC-N-20	20	6.4	15	30

5 **[00214]** To fabricate the capsules, water was mixed with PC with a mixer for 5 minutes until homogenous and followed by adding pre-mixed YE and CaL powders to the paste and mix for another 5 minutes until homogeneous. Then the fresh paste was cast into 2.5 cm cube mold. After hardening for 2 days, the cube was demolded and then cured in sealed plastic bag for another 5 days. At the age of 7 days, the cube was crushed into
 10 powder and sieved with a 150 μ m mesh. When the 3 different capsules were prepared, they were incorporated into PC paste together with RMC-B capsules to examine their influence on the strength of the matrix, respectively. The compositional configuration when incorporating different PC-N capsules are shown in table 16 below.

15 **[00215]** Table 16 – Compositional configuration of the PC pastes with RMC-B and PC-N capsules

Sample Name	PC (g)	Water (g)	RMC-B capsule (g)	PC-N capsule mix	PC-N capsule weight (g)
P-Dual80	1420	454.4	35	PC-N-80	150.6
P-Dual40	1460	467.2	35	PC-N-80	97.8
P-Dual20	1480	473.6	35	PC-N-20	71.4
P	1500	480	35	-	-

[00216] When preparing the pastes, the prepared RMC-B and PC-N capsules were first mixed with PC in a mixer for around 5 minutes. Afterwards, water was added and mixed for another 5 minutes until a homogeneous state was reached. The fresh pastes were
 20 cast into 50 mm cubic molds, sealed in an air-tight plastic to prevent water evaporation, and placed in ambient conditions (25°C, 80% RH). The cubic specimens were demolded after 2 days and cured under the same conditions for another 26 days.

Afterwards, the compressive strength of the specimens was tested at the age of 7, 14 and 28 days on a compression machine (ToniTechnik Baustoffprüfsysteme) operated at a loading rate of 55 kN/min.

[00217] The compressive strength results are shown in FIG. 28 (the 28-day strength of P-Dual80 was not tested). It can be seen from the figure that all these 3 mixes of PC-based nutrient capsules have decreased the strength of matrix dramatically. The decrease might be associated with 2 reasons. Firstly, the addition of large amount of nutrients in the capsules may compromise the strength of the capsules significantly therefore the nutrients capsules presented very low strength. Secondly, the nutrients might be leached out from the capsules during mixing and hydration, therefore the leached nutrients compromised the strength of the matrix. However, comparing the RMC-B-N capsules with the PC-N capsules here, when same amount of nutrients (45 g) was encapsulated in similar amount of cementitious materials (23.3 g of RMC), the incorporation of the RMC-B-N capsules in the matrix did not cause any strength loss. The results indicated that the carbonation treatment was a key procedure when fabricating the nutrients capsules as the dense shell formed in carbonation can prevent nutrients leaching out from the capsules.

[00218] Example 6: Commercial and Potential Applications

[00219] Concrete is the most widely used man-made materials, which has been globally produced at an amount exceeding 25 billion tonnes a year. In most developed countries, concrete maintenance and rehabilitation cost about 50% of the outlay on infrastructures. Deterioration of concrete infrastructure is associated with the formation of cracks. Thus, it is highly desirable to engage self-healing in concrete, i.e. the cracks being healed in natural environment without human intervention. The functionally graded bacteria capsules of the present disclosure are applicable as additives to concrete and other cementitious materials.

[00220] While the present disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the present disclosure as defined by the appended claims. The scope of the present disclosure is thus indicated by the appended claims and all changes

which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

1. A capsule incorporable into a cement-based material for repairing a crack in the cement-based material, the capsule comprising:
- 5 an inorganic particle having a surface and a core region,
wherein the core region houses a bacterial spore for repairing the crack;
or
wherein the core region houses a bacterial spore for repairing the crack
and the capsule houses a nutrient separate from the bacterial spore;
- 10 wherein the inorganic particle has a density and a strength which decrease in a direction from the surface to the core region, and
wherein the density at the surface sufficiently hinders penetration of an alkaline substance.
- 15 2. The capsule of claim 1, wherein the core region is porous.
3. The capsule of claim 1 or 2, wherein the core region comprises brucite and the surface comprises hydromagnesite or nesquehonite.
- 20 4. The capsule of any one of claims 1 to 3, wherein the core region houses the bacterial spore for repairing the crack and wherein the capsule is absent of a nutrient.
5. The capsule of any one of claims 1 to 3, wherein when the capsule houses the nutrient separate from the bacterial spore, the nutrient comprises yeast extract.
- 25 6. The capsule of any one of claims 1 to 5, wherein the bacterial spore comprises *Bacillus cohnii*, *Bacillus halodurans*, and/or *Bacillus pseudofirmus*.

7. A method of forming the capsule of any one of claims 1 to 6, the method comprising:
- providing a bacterial spore for repairing a crack in a cement-based material;
 - forming a paste comprising the bacterial spore and water or forming a paste
 - 5 comprising the bacterial spore, water and a nutrient;
 - allowing the paste to harden;
 - converting the paste which has hardened into particles; and
 - curing the particles in the presence of carbon dioxide to form the capsule.
- 10 8. The method of claim 7, wherein the paste comprising (i) the bacterial spore and water, or (ii) the bacterial spore, water, and the nutrient, further comprises reactive magnesia cement.
9. The method of claim 7 or 8, wherein curing the particles comprises curing the
- 15 particles in the presence of 10 vol% carbon dioxide and 80% relative humidity.
10. A nutrient capsule incorporable into a cement-based material for housing a nutrient which aids a bacterial spore for repairing a crack in the cement-based material, the nutrient capsule comprising:
- 20 an inorganic particle having a surface and a core region,
 - wherein the inorganic particle has a density and a strength which decrease in a direction from the surface to the core region, and
 - wherein the core region houses the nutrient and the capsule is absent of a bacterial spore.
- 25 11. The nutrient capsule of claim 10, wherein the nutrient capsule is formed of Portland cement.

12. A method of forming the nutrient capsule of claim 10 or 11, the method comprising:

providing a nutrient which aids a bacterial spore for repairing a crack in a cement-based material;

5 forming a paste comprising the nutrient and water;

allowing the paste to harden;

converting the paste which has hardened into particles; and

curing the particles in the presence of carbon dioxide to form the nutrient capsule.

10

13. A cement-based material incorporated with (i) the capsule of any one of claims 1 to 6, or (ii) the capsule of any one of claims 1 to 6 and the nutrient capsule of claim 10 or 11.

14. The cement-based material of claim 13, further comprising a nutrient incorporated in the cement-based material, wherein the nutrient is incorporated in:

the cement-based material and external to (i) the capsule of any one of claims 1 to 6, and when present, (ii) the nutrient capsule of claim 10 or 11.

15. The cement-based material of claim 13 or 14, wherein the cement-based material is derived from a cement comprising Portland cement.

16. The cement-based material of any one of claims 13 to 15, further comprising a calcium precursor, wherein the calcium precursor comprises calcium lactate, calcium acetate, calcium glutamate, or calcium formate.

17. A method of forming the cement-based material of any one of claims 13 to 16, the method comprising:

providing (i) a capsule of any one of claims 1 to 6, or (ii) a capsule of any one of claims 1 to 6 and a nutrient capsule of any one of claims 10 or 11; and

mixing the capsule, and when present, the nutrient capsule, with a cement to form the cement-based material.

30

18. The method of claim 17,
wherein providing (i) the capsule, or (ii) the capsule and the nutrient capsule
comprises:

5 (I) dispersing the capsule, and when present, the nutrient capsule,
homogenously in water to form an aqueous suspension prior to mixing the aqueous
suspension with the cement;

or

(II) preparing an aqueous solution comprising a nutrient, and mixing the
10 capsule, and when present, the nutrient capsule, into the aqueous solution to form a
mixture prior to mixing the mixture with the cement.

19. The method of claim 17 or 18, further comprising mixing a calcium precursor
with the capsule, and when present, the nutrient capsule, and the cement.

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20. The method of any one of claims 17 to 19, wherein the cement comprises
Portland cement.