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Espitia-Nery, Martin Eduardo; Corredor-Pulido, Dery Esmeralda;
Castaño-Oliveros, Paula Andrea; Rodríguez-Medina, Johan Andrey;
Ordoñez-Bello, Querly Yubiana; Pérez-Fuentes, Maikol Santiago

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Mechanisms of encapsulation of bacteria in self-healing concrete: review

Martin Eduardo Espitia-Nery, Dery Esmeralda Corredor-Pulido, Paula Andrea Castaño-Oliveros, Johan Andrey Rodríguez-Medina, Querly Yubiana Ordoñez-Bello & Maikol Santiago Pérez-Fuentes

Faculty of Engineering, Corporación Universitaria Minuto de Dios, Bogotá, Colombia.mespitian@yahoo.com, dery.corredor@uniminuto.edu, pcastanooli@uniminuto.edu.co, jrodrigu370@uniminuto.edu.co, qordonezbel@uniminuto.edu.co, mperezfuent@uniminuto.edu.co

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Abstract
Fissures in concrete structures result from structural deterioration and inadequate building processes, among other factors. Traditional *in situ*repair is often expensive and complex. For this reason, self-healing techniques have been developed including the use of bacteria that precipitate calcium carbonate to seal fissures. However, adding bacteria directly to the concrete matrix reduces bacterial survival. We present a review of different methods of bacterial encapsulation and their effects on fissure repair and concrete resistance. We argue that encapsulation of *Bacillussubtilis* in clay is the most promising method, increasing concrete strength by 12% and repairing fissures of up to 0.52 mm.

Keywords: encapsulation; bacteria; resistance; compression; self-healing concrete; cracks.

Mecanismos de encapsulación de bacterias destinados a la autorreparación de concreto: una revisión

Resumen
La aparición de fisuras en las estructuras de concreto es un fenómeno generado por el deterioro que suelen presentar este tipo de estructuras, procesos constructivos inadecuados, entre otros. La reparación *in situ* de las mismas se ha venido realizando desde hace muchos años, sin embargo, este tipo de mantenimiento es costoso y complejo de realizar en algunas ocasiones, razón por la cual se ha optado por técnicas de autorreparación, entre ellas el uso de bacterias que precipitan carbonato de calcio y sellan las fisuras; se ha demostrado que agregar las bacterias directamente (sin ningún tipo de protección) en la matriz del concreto minimiza la supervivencia de las bacterias en poco tiempo; este trabajo presenta una revisión de los diferentes métodos de encapsulación de bacterias que se han estudiado y cómo esto repercute en la eficiencia de reparación de las fisuras y en la resistencia del concreto, encontrando que la encapsulación de bacterias *Bacillus subtilis* en arcilla expandida es el método más prometedor para ser usado en este tipo de concreto (ancho máximo de fisura reparado de 0,52 mm y mejora de la resistencia del concreto del 12%).

Palabras clave: encapsulación; bacterias; resistencia; compresión; concreto autorreparable; fisuras.

1. Introduction

Concrete is one of the most utilised materials in the construction sector [1]. Due to several factors (including shrinkage by drying), volume changes may create internal tensions exceeding the tensile strength of concrete, thereby leading to fissures. Thermal stress and chemical reactions produced either by component materials or materials in contact with hardened concrete also generate structural fissures, which can appear at any stage of the useful life of a concrete structure [2,3,4]. Initially, fissures may only affect the appearance of a structure, but if not repaired in time they may expand and become cracks, allowing water, gases and other harmful substances to penetrate the concrete matrix, thus reducing the useful life of the structures [1].

The technique of *in situ* repair of fissures has become a less

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efficient alternative due to labour costs and overall budgets. Location of damage may also cause repair work to be virtually of impossible execution [5]. Therefore, for some years various techniques of concrete self-healing have been investigated, including low water/cement ratios (autogenous repair), where cement grains are only hydrated at a later stage by moisture or water penetrating fissures, resulting in their repair. However, autogenous repair is limited to cracks under 0.2 mm [5,6]. Another approach is the introduction of encapsulated healing agents such as polymeric compounds into the concrete matrix, with capsules breaking down in the presence of humidity, releasing the repairing agent [7]. However, polymeric compounds have a negative effect on the mechanical properties of concrete [8].

Biological self-healing alternatives have become increasingly popular thanks to research conducted since 2011 by the Dutch microbiologist Henk Jonkers at at the Technological University of Delft, inspired by the mechanisms of fractured bone repair. He developed a self-healing concrete that integrates encapsulated bacteria of the genus *Bacillus*, which precipitate calcium carbonate through bio-mineralisation thereby sealing fissures [9]. The technique is based on adding bacterial spores with encapsulated nutrients in the concrete matrix, which are then ruptured when in contact with water, humidity or oxygen, penetrating through any appearing fissure and inducing the process of bio-mineralisation.

Research has shown that adding bacteria and nutrients directly to the concrete matrix improves its mechanical properties [10,11], but bacterial survival after ten days of curing was between 1.9% and 7% [12,13] due to the high pH (between 12 and 13) and dry state of concrete. For this reason, encapsulation is essential to protect bacteria from the environment [8].

Various methods of encapsulation of bacteria have investigated factors such the material, size, distribution and quantity of capsules added in the concrete matrix. Capsules can increase survival of the bacteria but must be at the same time be strong enough to withstand the concrete mixing process, and fragile enough to break whenever cracks appear. Capsules must also be able to form a strong bond with the matrix to avoid negative effects on the mechanical properties of concrete [14].

Here we review the literature on bacteria-based self-healing concrete, with an emphasis on methods of encapsulation of bacteria and nutrients. We summarise each encapsulation method, their repair efficiency, the resulting compressive strength of concrete, deployed bacteria, and maximum width of repaired fissures in Table 1. Finally, we draw some conclusions from our evaluation and comparison of encapsulation methods.

2. Encapsulation of bacteria and nutrients

Previous studies have investigated bacterial species such as *Bacillus sphaericus*, *Sporosarcinapasteurii* and *Bacillus subtilis* [15], as well as aspects of encapsulation methods (size, material), nutrient type, and number of capsules required for the efficient repair of fissures. This review focuses on encapsulation techniques, including diatomaceous earth, lightweight aggregates (expanded clay), ceramsite, silica gel, hydrogel, polyurethane, graphite, metakaolin, among others. Encapsulation is one of the main factors affecting the compressive strength of concrete [14,16-18], asreviewed in the following.

3. Encapsulation methods

Although most research on self-healing concrete has not focused on encapsulation, some studies have made relevant contributions to the topic.

3.1. Diatomaceous earth

In 2011, Wang and colleagues [19] presented the only investigation so far of diatomaceous earth as an immobilisation agent to protect bacteria from the high pH (between 12 and 13) of concrete. The skeleton of diatomaceous earth is highly porous, chemically stable, inert, and irregularly shaped, with particle sizes ranging from 4 to 20 μm. Their results demonstrated effective bacterial encapsulation and sealing of cracks between 0.15 mm and 0.17 mm wide.

3.2. Light weight aggregates (expanded clay)

Another encapsulation method discussed in recent research is expanded clay [20-22], with diameters between 1-4 mm. A rate of 100% repair of fissures was obtained, although the maximum width of healed fissures varied. In Jonkers and Wiktor's study [20], maximum width was 0.46 mm [20], while Jonkers [17] replaced 50% of fine and coarse aggregate with light aggregate (expanded clay) loaded with bacteria and nutrients in the concrete matrix and healed fissures up to 0.15 mm. However, compressive strength decreased by 50%. Zhang et al. [21] managed to restore fissures with a maximum width of 0.79 mm by additionally coating expanded clay particles with a geopolymer layer of metakaolin and sodium silicate solution. A similar approach was adopted by Alazhari and colleagues [22], who coated expanded clay with a double layer of sodium silicate solution and cement powder to protect spores and nutrients in the concrete from leaching.

In 2015, Sierra, Mera and Jonkers [23] conducted the first application of self-healing concrete in a 3 m long irrigation canal stretch in Tungurahua province, Ecuador, using expanded clay impregnated with alkaline bacterial spores and their nutrients (calcium lactate and yeast extract). Initially, a concrete mixture of sand, gravel, type I cement, natural hemp fibres and expanded clay particles was tested in the laboratory at 28 days for compression and torsion, demonstrating improvements in resistance of 15.4% and 5.6% respectively. Torsion tests produced fissures with a width of 0.14 mm, which were exposed to water (simulating conditions faced by irrigation canal walls). Fissures were completely sealed after six weeks. Subsequently, the irrigation channel section was coated with the designed and tested self-healing concrete. Six months later, the canalhas not yet presented any signs of cracking, and therefore the self-healing properties of the concrete still await evaluation.

3.2.1. Lightweight aggregates (ceramsite)

Chen and colleagues [24] used ceramsite as a carrier of bacteria and nutrients. Ceramsite has similar characteristics to expanded clay, being able to retain liquids and preserve shape under compression or heating. In their study, bacteria and nutrients were immobilised independently, capsules were mixed with the cement paste, and samples were cracked up to their cross section through flexural loading. Results revealed repaired fissures with a width of 0.5 mm and an increase in resistance to torsion of 56% to 72% compared to other biological methods.

3.3. Geopolymers

Encapsulation or coating with geopolymers was developed in 2015 by Koster and colleagues [14], who used metakaolin and sodium and silicon activating fluids to coat bacteria and nutrients (calcium lactate) with a low shear granulator and a high-pressure fluid nozzle. Particle diameter varied between 1mm and 4mm in diameter. Subsequent leaching tests revealed that capsules had lost between 65% and 100% of the self- healing agent in three hours. Compression tests showed that coating loss occurred at relatively low loadings (between 1-3 N). As a rule, loadings over 10 N are required to damage the core of the self-healing agent. Results also indicated that the coating material interacts properly with the concrete matrix.

3.4. Polymers

Wang and colleagues [1] applied polyurethane and silica gel to immobilise bacteria and nutrients, which were then placed into glass tubes with a length of 40 mm and internal diameter of 3 mm. Precipitation of calcium carbonate was higher in the silica gel than in the polyurethane foam, since bacteria have a higher ureolytic activity when encapsulated in silica gel. However, polyurethane has more potential to be used in encapsulation methods due to its higher resistance recovery (60%) and lower water permeability coefficient (10-10 - 10-11 m/s).

In 2014, Wang and colleagues [25,26] usedhydrogels to encapsulate bacteria and nutrients in the concrete matrix. The advantage of this method is that hydrogels exhibit water absorption and retention properties allowing capsules to retain moisture, which facilitates bacterial activity and precipitation of calcium carbonate. Their results revealed a rate of fissure repair between 40% to 90%, a maximum fissure width of 0.5 mm and a reduction in permeability of68%. Encapsulation with pure hydrogel resulted in water absorption of approximately 70% and 30% after 12 h and 24 h respectively [25], implying that contact time with water can be reduced due to the ability of hydrogels to absorb air moisture [26].

3.5. Microencapsulation

Microencapsulation techniques based on melamine have also been used to coat bacteria. In 2014, Wang and colleagues [18] employed a micro-encapsulation process based on a patented polycondensation reaction. Repair by bacteria was measured as the ratio of healed fissure area to initial fissure area. The experiments achieved ratios between 48% and 80%, with the highest values observed when samples were exposed to wet and dry cycles (alternating a constant water jet on specimens, followed by a dry stage, and so on). Maximum width of repaired fissures was around 0.97 mm, and addition of microcapsules also reduced capillary water absorption. However, results were not entirely positive because addition of microcapsules at 3% and 5% decreased compressive strength of concrete by 15% and 34% respectively.

3.6. Other **methods**

Another recently developed method is the use of graphite nanoplatelets (GNP) for encapsulation by Khaliq and Ehsan in 2016 [27]. Results indicated an increase in compressive strength of 9.8% due to the nanosize of particles, which act as filling material and ensure uniform distribution along the concrete matrix. Maximum width of healed fissures was 0.38 mm. The study also investigated lightweight aggregates (expanded clay) with even more positive results. Maximum width of repaired fissures was 0.52 mm, closely matching the values reported byJonkers and Wiktor in 2011, while resistance increased by 12% compared with non-bacterial concrete.

Table 1 summarises the different methods of encapsulation as well as their main associated results.

4. Discussion

Table 1 shows that encapsulation of *Bacillus sphaericus* bacteria in melamine microcapsules achieved the highest fissure repair efficiency (0.97 mm), followed by encapsulation of *Bacillus cohnii* bacteria in expanded clay coated with a layer of geo-polymer (0.79 mm). However, resistance to compression decreased by 15% for the former, while no tests were carried out for the latter. Further investigations into this issue are therefore crucial for a discussion of the relative merits of diverse concrete self-healing methods.

Use of ceramsite as encapsulation material increased resistance to torsion by a value between 56-72% compared to other methods, with a maximum width of repaired fissures of 0.5 mm. However, further tests of resistance to compression are necessary for a full evaluation of the method.

The use of hydrogels as an encapsulation method must be further investigated due to their water absorption and retention properties, which may reduce the need of water in self-healing processes.

Despite the increase in resistance to compression it achieves, the use of polyurethane and silica gel to immobilise bacteria only heals relatively narrow fissures. The same was true for graphite nanoplatelets and expanded clay, achieving increases in compressive strength of respectively9.8% and 12%compared to non-bacterial concrete, but a maximum width of repaired fissures of only 0.38 mm.

Table 1.
Encapsulation methods, descriptions and results.

Bacteria	Encapsulation method	Bacterial culture method	Bacterial count	**Resistance to compression (28 days)	Maximum width of repaired fissure (mm)	Time to appearance of fissure/method	Variation in resistance to compression	Ref.
Bacillus sphaericus	Diatomaceous earth	Yeast extract and urea	10^9cells /ml	*	0.15 – 0.17	14 days/three-point flexion	*	[19]
Bacillus alkalinitrilicus	Expanded clay	Calcium lactate and yeast extract	1,7x10^5g^{-1} bacterial spores per particle	*	0.46	56 days/controlled tension	*	[20]
Genero bacillus	Expanded clay	Calcium lactate	1.7x10^5 g^{-1}bacterial spores per expanded clay particle	*	0.15	60 days/controlled tension and compression	Resistance to compression decreased by 50%	[17]
Bacillus cohnii	Expanded clay coated with geopolymer layer	Calcium lactate and yeast extract	5,2x10^8cells/cm^3	34 MPa	0.79	28 days/controlled tension	*	[21]
Bacillus pseudofirmus	Expanded clay coated with double layer of sodium silicate solutionand cement powder	Buffered lysogeny broth/calcium acetate, yeast extract, and dextrose	4.1x10^9spores/g	*	*	28 days/division test	*	[22]
Alkaliresistant bacteria	Expanded clay	Calcium lactate and yeast extract	*	30 MPa	0.14	42 days/three-point flexion	Resistance to compression increased 15.4%	[23]
Bacillus subtilis	Expanded clay	Nutrient broth	2.8x 10^8cells/ml	4000psi (27.6 MPa)	0.52	28 days/compression	Resistance to compression increased by 12%	[27]
Sporosarcina pasteurii	Expanded clay	Nutrient broth, urea, $CaCl_2$	10^6 cells/ml	34.9MPa	*		Resistance to compression increased by 20%	[28]
Bacillus mucilaginous	Ceramsite	Beer yeast	*	*	0.5	28 days/three-point flexion	Resistance to flexion increased by 56% to 72%	[24]
Bacillus sphaericus	Polyurethane	Yeast extract and urea	10^9cells /ml	*	0.35	14 days/three-point flexion	Resistance to compression increased by 60%	[1]
Bacillus sphaericus	Silica gel	Yeast extract and urea	10^9cells /ml.	*	0.35		Resistance to compression increased by 5%	[1]
Bacillus sphaericus	Hydrogels	Yeast extract and urea	10^9spores /ml	*	0.5	28 days/tension	*	[25]
Bacillus sphaericus	Melamine microcapsules	Minimal basal salt (mbs) / urea	109 cells/g	50-80MPa	0.97	28 days/tension test	Resistance to compression decreased by 15%	[18]
Bacillus subtilis	Graphite nanoplatelets	Nutrient broth	2.8x 10^8cells/ml	4000psi (27.6 MPa)	0.38	28 days/compression	Resistance to compression decreased by 15%	[27]

*Data not reported
**Compression resistance tests on cylindrical or prismatic tubes were performed at room temperature.
Source: The Authors.

The use of expanded clay improves compressive strength of concrete also due to the selected bacteria. In 2017, Balamand colleagues encapsulated *Bacillus subtilis* in expanded clay and obtained an increase in compressive strength of 20% and a maximum width of repaired fissures of 0.52 mm. Therefore, expanded clay is a viable method and its possible applications should be further investigated.

5. Conclusions

The best performing bacterial encapsulation methods as measured by fissure repair efficiency are microencapsulation based on melanin, followed by expanded clay coated with a layer of geopolymer consisting of metakaolin and sodium silicate solution, producing maximum repair widths of 0.96 mm and 0.79 mm respectively. As for compressive strength, graphite nanoplatelets, followed by either expanded clay or polyurethane, result in increases in resistance of 9.8%, 12-20% and 60% respectively.

Encapsulation of *Bacillus subtilis* bacteria in expanded clay is the most promising method, with a maximum repaired fissure width of 0.52 mm, and an improvement of concrete strength by 12%.

From our review it can be concluded that the greater the area of repaired fissure, the lower the compression resistance of concrete, since capsules introduced at high percentages do not confer the same resistance as the materials they replace, such as sand, gravel and cement mix.

It is important that future research on self-healing concrete assessesboth the efficiency of fissure repair and the compressive strength of concrete, to assist in analyses of viability of methods, design, and applications of concrete mix.

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M.E. Espitia-Nery, is a BSc. in Physical Engineer, Sp. in Production and Operations Management, MSc. in Industrial Engineering, MSc. in Business Administration, and currently a student in the MBA program at the Universidad de los Andes, Colombia. He has 10 years of experience in teaching, research and management of strategic plans, specialized in companies in the services and manufacturing sector in areas of production, operations and R&D + i. Consultant and lecturer in countries such as Spain, Brazil, Mexico, among others, in topics related to engineering, science and materials technology. He is currently a principal investigator in the GEIIC group.
ORCID: 0000-0001-5626-3051

D.E. Corredor-Pulido, is a BSc. in Metallurgy Engineering, MSc. in Metallurgy at the Universidad de Concepción in Chile, and a researcher in the GEIIC research group. She is currently a full professor at the Corporación Universitaria Minuto de Dios, Colombia, Faculty of Engineering, a national and international speaker, and expert in ONAC scanning electron.
ORCID: 0000-0003-3148-9219

P.A. Castaño-Oliveros, is a student in the BSc in Civil Engineering at the Corporación Universitaria Minuto de Dios, Colombia. She is currently a member at SINCE, the Sustainable Construction and Structures Seedbed. He has delivered presentations on composite materials at national events.
ORCID: 0000-0002-3492-7619

J.A. Rodríguez-Medina, is a student in the BSc in Civil Engineering at the Corporación Universitaria Minuto de Dios, Colombia. He is currently a member at SINCE, the Sustainable Construction and Structures Seedbed.
ORCID: 0000-0001-8446-9364

Q.Y. Ordoñez-Bello, is a student in the BSc in Civil Engineering at the Corporación Universitaria Minuto de Dios, Colombia. She is currently a member at SINCE, the Sustainable Construction and Structures Seedbed.
ORCID: 0000-0002-3807-9821

M.S. Pérez-Fuentes, is a student in the BSc in Civil Engineering at the Corporación Universitaria Minuto de Dios, Colombia. He is currently a member at SINCE, the Sustainable Construction and Structures Seedbed.
ORCID: 0000-0003-3148-6472