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Microbial loading and self-healing in cementitious materials: A review of immobilisation techniques and materials

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ABSTRACT

Concrete has been a material of choice when it comes to building materials for decades. However, concrete has a number of challenges in which a major challenge being microcracking leading to excess damage and wastes. The development and advancement of self-healing technology throughout the past decade have seen the popular use of immobilization as a way of protecting bacteria from the harsh environments found in cementitious materials. This paper reviews the materials used for immobilization, categorising into organic materials and inorganic materials, and investigates the various immobilization techniques used to immobilize bacteria into polymeric structures and porous materials. The study evaluates the key findings in literature surrounding immobilization materials and methods as well as highlighting possible alternative sustainable materials and methods including waste/by-product resources. It was found that inorganic materials were superior to organic material in terms of self-healing and mechanical properties, with nanomaterials producing the highest crack closure of 1.20 mm. Various immobilization techniques efficiency was tested comparing microencapsulation, vacuum impregnation and adsorption methods. Further studies are needed to understand the relationship between carrier materials and cementitious matrix and explore the possible use of nanomaterials as a way of uniformly distributing bacteria in cementitious matrix.

1. Introduction

Concrete has shaped modern day infrastructure worldwide. Not only it is cost-effective when compared to other construction materials (Fig. 1), but it is extremely versatile in the design, non-combustible, low cost of materials and characterized by high compressive performance, making it a preferred option as a construction material globally. One of the greatest challenges with concrete is the relatively low tensile strength, which can cause the development of microcracks. Previous research stated that cracking is predetermined due to the nature of concrete[15,20,13]. [15] suggested cracks up to 0.30 mm are unavoidable, even in some standards, namely BS 8110, cracks up to 0.30 mm are considered acceptable. Cracks usually occur prior to concrete hardening due to a combination of structural, physical, and chemical causes. Cracks occurring after concrete hardening are often linked to plastic cracking or movement caused by construction activity, e.g., accidental impact. Common causes of cracking in concrete include plastic shrinkage, freeze thawing, compressive and tensile stress to list a few. Microcracks of up to 0.30 mm are generally considered not to pose a significant threat to structural integrity, however, they can significantly affect the structural durability, as the microcrack may represent a point of infiltration for harmful substances, which can lead to the oxidation of steel reinforcements and deterioration of the concrete matrix. Available solutions to remediate microcracks include: i) injection grouting [72,17] and ii) epoxy resin grouting[72]. Maintenance and repairing of microcracks leads to a very laborious and expensive process with repercussions on different construction sectors[55]. Indeed, not only it put financial strains on the industry as approximately 40 % yearly construction budget in the UK is devoted to maintenance and repair works, but it also impacts the environment as ordinary Portland cement (OPC) alone contributes to approximately 7 % of CO_2 emissions [40]. Extending the service life of concrete or any other systems can minimize the need for additional constructions while decreasing the maintenance cost and GHG and VOCs emission[75], and self-healing approach is a pathway to circular economy systems[8,26,98].

Self-healing was first discovered by the French academy of science in

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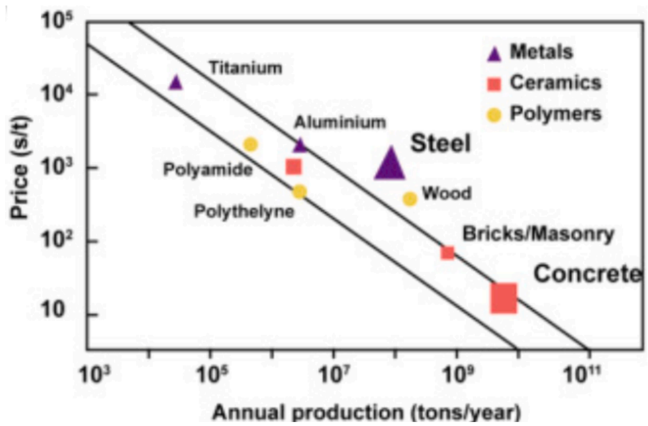

Fig. 1. Price of construction materials relative to the amount produced annually [99].

1836. Self-healing concrete refers to the ability of concrete to detect and repair cracks autonomously. Self-healing can be categorised into two groups: i) autogenous healing and ii) autonomous healing. Autogenous healing is known as the natural healing because concrete utilises the components originally found in it to promote cracks healing[18]. Engineering the healing mechanism by introducing foreign elements into concrete is known as autonomous healing[18]. In the technical literature, such elements are polymers, fibres, minerals, chemicals or bacteria. The use of self-healing concrete can provide economic, environmental, and social benefits as it helps reducing the (i) cost of maintenance and repair, (ii) production of CO_2 and finally, (iii) disruption to transportation system. The present review paper will focus on the autonomous healing mechanism, in particular the use of bacteria as a healing agent. In recent years microbial induced self-healing has become a popular area of research [40,45,46,63,77,81,83,67]). This technology utilizes the production of $CaCO_3$ to heal cracks by using microbial material as an additive to promote the healing phenomenon. This is achieved via two main methods: (i) metabolic conversion of organic acids, which utilises the microorganisms as catalysts [40]and (ii) ureolytic bacteria. In the latter method, bacteria produce urease enzyme, which hydrolyses urea to two moles of ammonium and one mole of carbonate [89].

Within this field of research, academics have identified major challenges related to the use of bacteria in concrete. Bacteria need favourable conditions to grow and survive, and the concrete mixing process, the high alkaline conditions within the concrete pores and the relatively dry environment once the concrete is poured pose a great threat to their survival[40,57,97]. A review conducted by Mohamed et al. [51] highlights the importance of carrier material. Jonkers et al. [40] observed that the longer the bacteria are inside the cementitious material, the lower their viability. The study pointed out that the viability can decrease up to 99.97 % from day 9 to day 135. Also, multiple researchers agree that directly applying bacteria into the cementitious material will lead to their killing [40,95,45,46,48,63]. This dramatic decrease in bacterial viability and its link with the time factor can be justified by the fact that once the microstructure of the concrete has developed, the pores become as small as 0.5um. Considering that the size of the bacteria is 1-3um and of the spores is around 1um, this suggests that as the microstructure develops the bacteria are crushed (Wang et al., 2014). As can be clearly deduced, immobilizing the bacteria and protecting them from harsh environments present in the concrete is of fundamental importance in order to preserve their viability.

Since the self-healing mechanism in concrete was observed for the first time[31], this technology has seen important developments. Some milestones are represented by the experimental work conducted by Wagner [74] who observed 0.05 mm-0.10 mm self-healing effect in cement lined pipes after healing for 50 days and the identification of four mechanisms including i) Hydration, ii) CaCO3 formation, iii) swelling and iv) block of debris and impurities proposed by [21]. Finally, it is worth mentioning the work done by Jonkers [37] who studied the potential of immobilizing bacteria in cementitious materials providing superior healing properties.

Currently, scholars are interested in developing optimized immobilization methods, through selection of suitable materials to act as a carrier and using coating materials to improve retention of bacteria. The aim of this review paper is to provide an overview of the current state of research on immobilization materials and methods for bacteria used in self-heling concrete, highlighting key findings and inconsistences in knowledge within the field. The review specifically 1) focuses on the detailed categorization and evaluation of organic and inorganic materials and various immobilisation techniques, such as microencapsulation, vacuum impregnation, and adsorption methods; 2) goes into the drying processes crucial for effective bacteria immobilisation; 3) provides a comparative analysis of different materials and methods/techniques, highlighting their efficiency in terms of self-healing capabilities; 4) highlights a promising future direction in which immobilisation should go down in the use of nanoparticles such as nanocellulose, with focusing on the nano-properties of compatibility of cementitious matrix, carrier material as well as the precipitate produced. Thus, this review covers a systematic and comprehensive evaluation of immobilisation supply chains from various materials to techniques to crucial issues of related new developments, and their correlations with efficiency, aiming at suggesting possible alternatives to improve methods of immobilization.

2. Materials used in immobilization

Immobilization is fast becoming a key technique in self-healing materials to protect microorganism for Microbial Induced Calcium Carbonate Precipitate (MICP)[9,4,35,36,45,46,57,64,72]; Wang, et al., 2014; [78,77,81,86,91,100]. The term immobilization for bacteria self-healing concrete refers to a preventative measure that stops the free movement of the bacteria, both in the form of vegetative cells or spores. This can be achieved using multiple methods which will be critically discussed in the present paper. The most common approach comprises enclosing the bacteria in a shell-like structure or fixating the cells to another material using, to name a few, (i) adhesion, (ii) covalent bonding[66]and (iii) ionic bonding[66]. Fig. 2 shows the most prominent techniques used to transport bacteria into immobilization materials, as well as the drying processes to remove moisture from the immobilization materials. In order to achieve a successful self-healing product, focus must be placed on the loading ability of the techniques as well as the ability of the immobilization process to preserve the bacteria from premature activation. Various carrier materials have been studied, for instance: (i) glass [44], (ii) expanded perlite[97], (iii) hydrogel[19,76,79], (iv) sodium alginate[35], (v) natural fibres [57,64,56], (vi) Alkali activated lightweight aggregate (LWA)[4,45,59] and (vii) recycled aggregate[47,48]; Xu et al., 2024). The following sections provide a detailed discussion of immobilization materials grouped into organic and inorganic carrier materials. The sections will highlight their influence on self-healing abilities and suitability to be used as a carrier material.

2.1. Organic materials as carriers for self-healing cementitious materials

Organic materials play a crucial role in the immobilization of bacteria, by providing sufficient protection and structural support to the cementitious matrix. Multiple organic immobilization materials have been studied for self-healing, e.g. hydrogel[76], melamine[79], Chitosan[78], natural fibres[57,64], sodium alginate[35]. Fig. 3 and Table 1 provide examples of different carrier materials. Organic materials are characterized by their large amount of carbon atoms combined to other atoms or elements. Often carbon atoms are covalently linked to

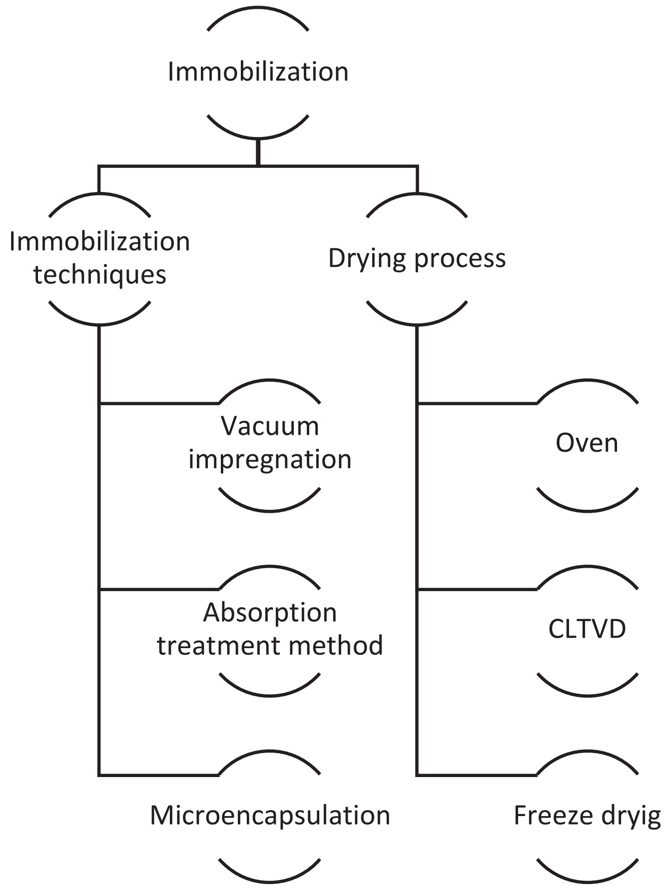

Fig. 2. Immobilization system illustrating techniques and drying options (CLTVD=Controlled low temperature vacuum dehydration).

nitrogen, oxygen, or hydrogen. Organic carrier materials, such as natural fibres and hydrogel, are relatively cheap, sustainable, and non-toxic. They are compatible with bacteria and highly water absorbent, which are favourable for the germination of spores. Additionally, they can provide fibre bridging, which restrict cracks from increasing in size and keeps both sides of the crack in contact for a more effective healing. However, natural fibres can lose strength in high alkaline environments [85], which is not favourable for self-healing composites. There are also concerns about their mechanical properties within cementitious materials[64]. Thus, coating fibres may address these drawbacks by improving interfacial bonding and resistance to the harsh environment in cementitious material. Microencapsulation using sodium alginate and melamine, provides a protective barrier between the bacteria healing agent and harsh concrete environment. Depending on the design microcapsules can have high loading capacity. However, the high cost and complicated processing procedures may deter widespread application. The summary of the pros and cons found in Table 2.

An ideal candidate as a carrier material for bacteria is represented by hydrogel, a hydrophilic material with high water absorption and porosity. The specific features of hydrogel help create a favourable environment for spore germination as water and oxygen are abundant [76]. As visible in Table 1, the hydrogel structure allows for water to be absorbed to facilitate the bacterial activity to produce $CaCO_3$, hence 0.50 mm wide crack widths can be sealed within 28 days. In a similar study conducted by Wang et al. [78], chitosan hydrogel was found only able to heal 0.25 mm cracks. This means that chitosan displays lower self-healing abilities in terms of crack width sealing when compared to other organic materials with similar properties such as hydrogel and melamine. However, chitosan hydrogel was able to reduce water flowing through the crack by 87 % within 28 days of healing[78], whereas hydrogel was only able to reduce water permeability by 68 % within the same timeframe[76]. This difference in water tightness may be attributed to chitosan high alkaline resistant being able to absorb highly alkaline solution, thus having better sealing properties when compared to conventional hydrogel. Table 1 shows that using melamine as a immobilization material, it is possible to heal crack widths of 0.97 mm. The level of healing observed in chitosan is comparable to that achieved

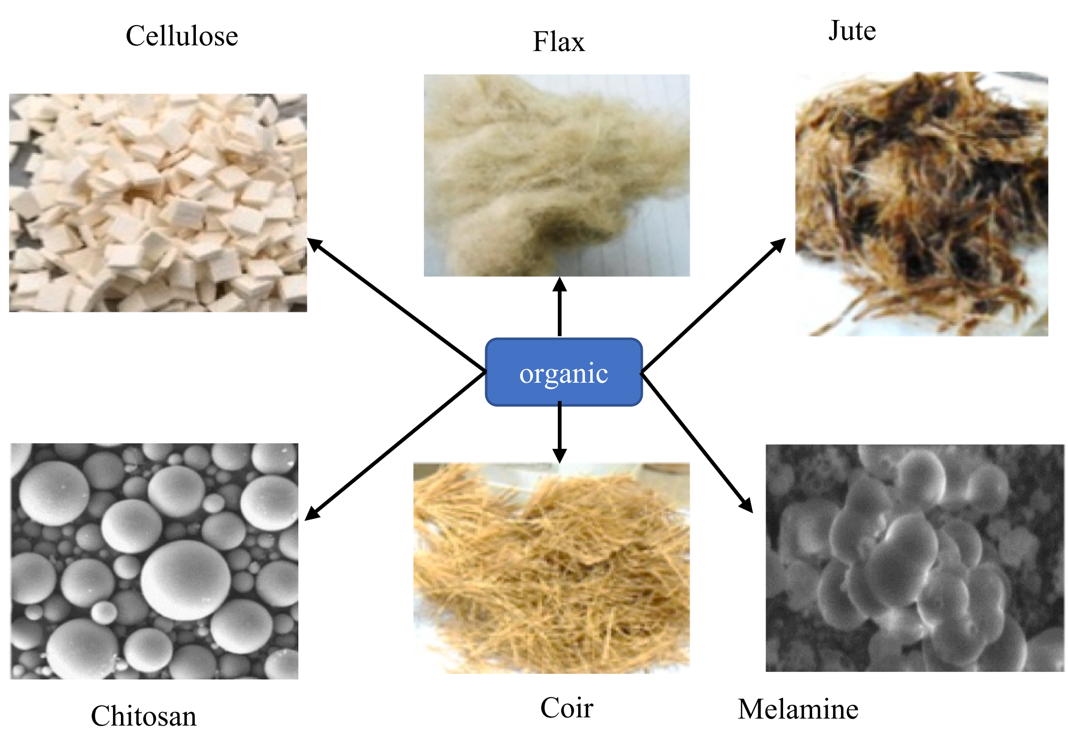

Fig. 3. Display of organic immobilization materials used for self-healing purposes compiled from previous reports [64,34,57,61]).

Table 1
Summary of various organic carrier materials used in the technical literature and the influence on self-healing performance of cementitious materials.

Immobilized material	Self-healing performance	Reference
Hydrogel	• Ability to heal crack widths of up to 0.50 mm in 28 days of healing. • Able to produce approximately 10 time more CaCO3 precipitate volume than autogenous healing.	[76]
Melamine	• Ability to healing crack widths up to 0.97 mm in 56 days of healing. • Improvement in water permeability of 10 times that of the reference samples.	[79]
Chitosan	• Ability to heal crack widths of up to 0.25 mm in 28 days of healing. • Plain Chitosan had the ability to decrease water flow by 80 %. • Chitosan with spores were able to further have a reduce water flow of 87 %.	[78]
Flax	• Ability to heal crack widths of up to 0.40 mm in 28 days of healing. • Regained 95.10 % of compressive strength • Improvement in UPV (%) of 21.6 % after 56 days healing.	[57]
Jute	• Ability to heal crack widths of up to 0.40 mm in 28 days of healing. • Improvement in UPV (%) of 14.1 % after 56 days healing. • Regained 98.40 % of compressive strength	
Coir	• Ability to heal crack widths of up to 0.40 mm in 28 days of healing. • Improvement in UPV (%) of 4.1 % after 56 days healing.	
Sodium Alginate	• Ability to obtain 97.60 % healing ratio after healing for 7 days. • Significant decrease of 70 % in ultimate load when compared to control samples.	[35]
Cellulose fibres	• Ability to heal crack widths of up to 0.07 mm after 28 days of healing. • Increase in tensile strength of 7.80 % • Able to achieve 12.04 % and 10 % self-healing of cracks after pre-cracked at 14 and 28 days respectively.	[64]

Table 2
Pro's and con's of organic carrier materials.

Carrier	Pros	Cons
Hydrogel	o Moisture retention o High water absorption o Biocompatible	o Complex immobilisation o Cementitious compatibility
Natural fibres	o Biocompatible o Moisture retention o High water absorption o Low cost o Biodegradable o Environmentally friendly o Fibre bridging	o Degradation o Variation in quality and mechanical properties o Bacteria exposed to cementitious matrix
Melamine	o Moisture retention o Controlled release of bacteria o Biocompatible	o Cost o Non-biodegradable o Compatibility issue with cementitious matrix o Nutrients can't be encapsulated with spores unless in powder form
Sodium alginate	o Controlled release of bacteria o Biocompatible o Moisture retention and absorption o Protective barrier o Non-toxic	o Nutrients can't be encapsulated with spores unless in powder form o Water sensitivity (shrink and swelling)

by autogenous healing as demonstrated in the previous research [79].

The use of natural fibres in concrete is common and has been adopted for decades [5]. Rauf et al. [57], Rajesh and Sumathi [56] and Singh and Gupta [64] have studied the use of natural fibres as carrier for self-healing concrete purposes. Natural fibres have many favourable properties, especially highly porous and complex network microstructures, which make them great options for immobilization materials. In addition, Natural fibres are an abundant renewable resource, they have high water absorption and are characterized by good mechanical properties [49]. Because of the specific features mentioned earlier, they provide a source of water to improve internal curing and generate a bridging effect to limit crack widths. [72] discussed that fibres can contribute to a more complete healing as they provide a restrictive force on cracks, keeping them from growing wider. Rauf et al. [57] used coir, flax and jute fibres as immobilization materials and found that, regardless of the type of fiber used, the maximum crack width healed was 0.40 mm. Similarly, the compressive strength regains varied depending on the fibres used, with the highest compressive strength regained observed in jute fibres (98.40 %). However, [56] led to an increase in compressive strength of 23.04 %, 7.51 % and 10 %, further increasing when immobilized with bacteria to 29.40 %, 10.88 % and 13.61 % for coir, flax and jute respectively. This is due to the bacteria leaking from the fibres and CaCO3 produced filling the pores acting as filler materials, hence the improvement in strength. However, [56] did not see strength regained similar to [57]; they only achieved 37.98 % with immobilized jute fibres. This can be attributed to the healing conditions as [57] subjected to water curing, whereas [56] subjected to soil exposure. It can be seen that, water immersion increases strength recovery of self-healing specimens, due to the presences of liquid water, whereas in soil, the water can be absorbed by the soil particles, and bacteria within the soil competing with the bacteria placed in the self-healing specimen (as seen in Fig. 4). Additionally, Palin et al., 2017, used seawater to heal cracked specimen and found 95 % of original water tightness was achieved for crack widths of 0.40 mm. Which suggests significant healing, whereas using soil healing condition were only able to achieve healing efficiency of 10–58 % using the same carrier material[27]. This highlights the importance of healing conditions for effective self-healing. However, it may also be due to various factors that influencing the self-healing mechanism, which is covered in [51] review paper. Singh and Gupta [64] used cellulose fibres as carriers for bacteria and observed a negative

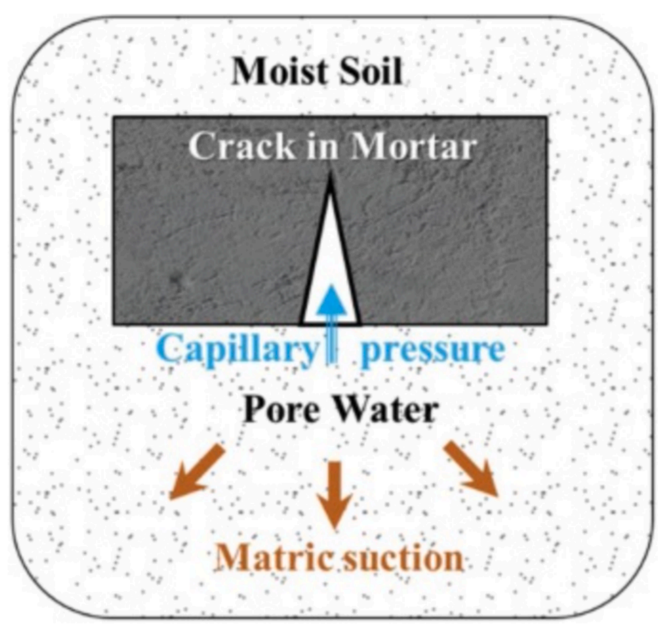

Fig. 4. Schematic of soil healing condition influence on crack through capillary pressure and matric suction [27].

trend in terms of compressive strength regain the and self-healing. In particular, the latter failed to meet expectation of sealing crack widths of 0.07 mm. Their finding suggests that insignificant healing effect could be attributed to the immobilization method or leakage of the bacteria during mixing. The use of cellulose fibres as a carrier requires more research for the use as immobilizing bacteria. The key areas of focus include preventing bacterial leakage and shielding the bacteria against concrete pore solution infiltration due to high alkalinity.

Organic polymers have also been proven to be effective carrier materials for bacteria. Melamine, given its resistance to high pH and sensitivity to humidity[79]is deemed to be a good potential carrier for bacteria. A favorable trait for carrier materials is their adaptability to the concrete environment. Melamine possesses a unique feature which allows it to become flexible when humidity is high and brittle when humidity is low[79]. Because of that, microcapsules breakage can be avoided during the mixing of cementitious material due to the present of water making it flexible. Once the cement paste hardens, the microcapsules will become brittle due to the lack of moisture and humidity in harden cement paste. Thus, amongst all the organic carrier materials, melamine can heal a maximum crack width of 0.97 mm and reduce the water permeability of cementitious samples to 10 times that of the control specimens.

Sodium alginate is another organic polymer used as carrier material. Because of its good water absorption and non-toxic nature, sodium alginate represents another option for immobilizing bacteria. Intarasoontron et al. [35], studied the use of Sodium alginate and were able to observe 97.60 % self-healing in the concrete specimens. However, the addition of the carrier material resulted in a decrease of the ultimate load up to 70 % compared to the reference sample[35]. Similar trends were also confirmed by Wang et al. [79]. Decreases of 15 % up to 34 % were observed, when 1–5 % microcapsules were introduced into the microstructure. As well as Huang and Ye [33] found that the increase in capsules caused a decrease in mechanical properties. This decrease in mechanical performance can be attributed to various factors including adhesion between microcapsule and surrounding cementitious matrix, morphology, and dimensions of the microcapsule as well as their impact on the compactness of the cementitious matrix.

To avoid negative effects on the mechanical properties caused by the presence of the carrier material, academics developed different methods involving coating and use of compatible carrier materials to concrete to improve bonding between the cementitious matrix and the carrier materials.

2.2. Inorganic materials as carriers for self-healing cementitious materials

Inorganic materials are characterized by the absence of carbon-hydrogen bonds. The use of inorganic materials has emerged as a promising alternative to organic carrier materials. Table 4 and Fig. 5 summarize inorganic materials explored in the technical literature and their observed self-healing performance. Inorganic carrier materials are widely used in research due to advantages they provide such as being widely available, low cost, and possessing highly porous networks, which make them excellent candidates for carrier materials (See Table 3). However, the use of porous inorganic carriers has drawbacks, primarily due to the variability in performance (X. [84]stemming from an increase in interfacial zones when utilising recycled materials, such as recycled concrete aggregate. Additionally, larger pores may not provide adequate protection to the bacteria, allowing the highly alkaline pore solution to penetrate the pores and possibly wash out of the bacteria due to weak bonding between the bacteria cell wall and porous inorganic material (e.g., van der Waals forces, and hydrogen bonding). A solution to these challenges include using coating materials to protect

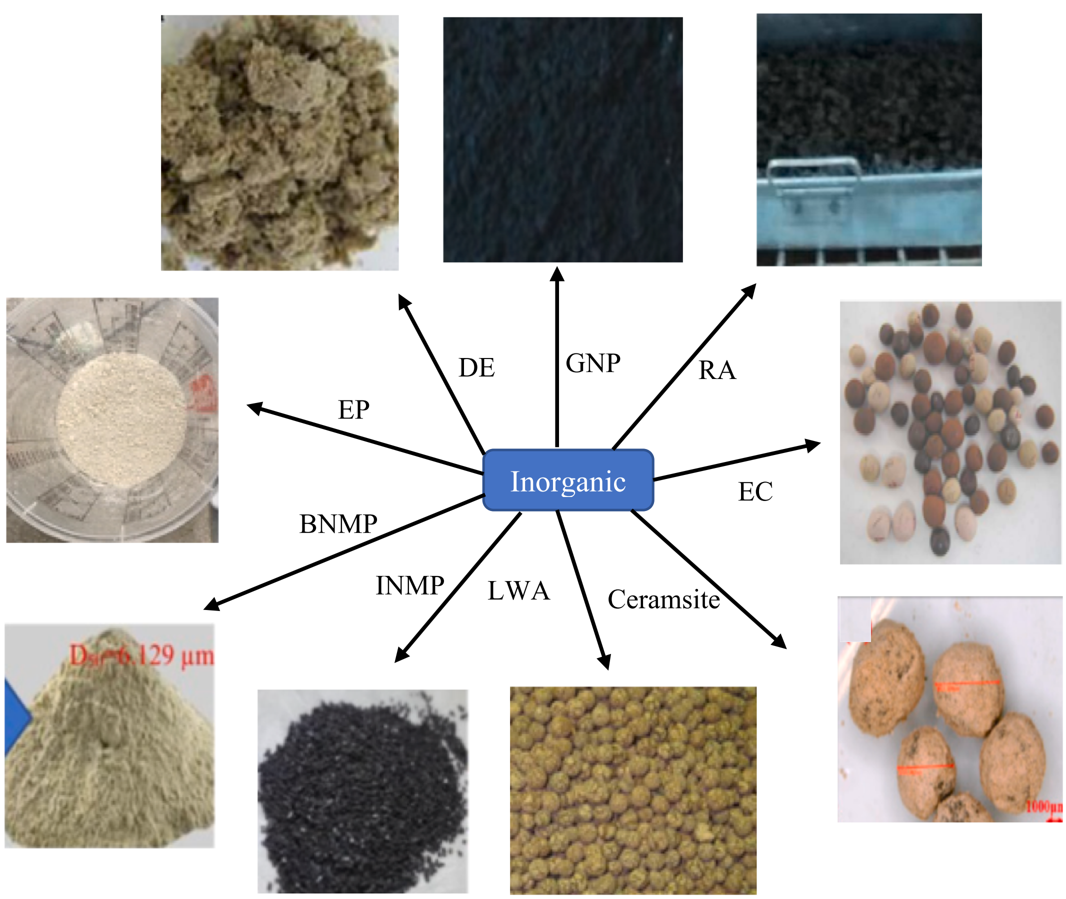

Fig. 5. Display of inorganic immobilization materials used for self-healing purposes compiled from previous workers [82,43,47,3,63,12,65].

Table 3
Pro's and con's of inorganic carrier materials.

Carrier	Pros	Cons
Expanded perlite	o Light weight o Highly porous o Low density o High water absorption	o Distribution challenges o Potential point of weakness o Bacteria leaching
Recycled concrete aggregate	o Low cost o High porosity o Compatibility with cementitious matrix o Water absorption o Waste reduction o Resource conservation	o More interfacial transition zones (weaker material) o Variable quality and composition o Bacteria leaching o Limited durability
Zeolite	o High porosity and surface area o Improves durability of cementitious material o High water absorption	o Delay strength development o Reduces workability o Bacteria leaching
INMP	o Uniform distribution o Biocompatibility o Improving mechanical properties o High adsorption ability o Low toxicity	o Cost o Use of hazardous chemicals and toxic by-products

the bacteria within pores, which is discussed in this paper.

According to Erşan et al. [22], the introduction of Diatomaceous Earth (DE), Expanded Clay (EC) and zeolite could lead to a decrease in compressive strength of mortar specimens of 2 %, 1 % and 20.82 %, respectively. An important factor to be considered when selecting a carrier material is its compatibility with the cementitious matrix. The use of zeolite as carrier in self-healing applications is hindered by the dramatic decrease of the concrete compressive strength. [6], investigated the use of zeolite in cementitious material and found a 3.90 % decrease in compressive strength. However, when immobilised with bacteria compressive strength increased by up to 13.77 %. [86] used EC as a carrier for bacteria and were able to heal cracks up to 0.46 mm in 100 days. The healing was due to the porous nature of the EC particles. However, S. [29] reported the pores of EC particles to be approximately 10-100um in size.

While theses pore sizes are considerably larger than bacteria cells which are approximately 1-3um[76], they still provide restricted volume for immobilization and growth of bacteria when healing process initiates.

Expanded Perlite (EP) is another popular inorganic material used for immobilization due to its porous structure and availability, which makes it a promising material as a bacteria carrier. [36] studied the use of EP as a carrier and found that it had great water absorption capacity under vacuum and atmospheric pressure of 275 % and 366 % in 24 h respectively. Also, [1] study the use of EP and found the water absorption capacity of 146 %, which suggest EP have great loading capacity. However, [36] in the same study pointed out that EP loaded with bacteria was only able to heal cracks of up to 0.27 mm and the adoption of EP reduced the permeability by approximately 46.15 %. In a separate study, [1] found that the use of EP as carrier for self-healing concrete led to a 68.18 % decrease in surface absorption. Within 120 min, specimens containing EP with nutrients and spores were able to achieve similar level of water surface absorption as uncracked specimens. Yan et al., 2023 Yan et al., 2023, investigated the use of S. Pastuerii immobilised into EP., Thethis study foundwas able to find an increase in the maximum crack width healed of 0.71 mm. Additionally, the material exhibited anlong with impressive regained flexural strength of 102.4 % after healing in water immersion conditions, suggesting the material became stronger than original pre-cracked. Diatomaceous Earth (DE) is a highly porous and lightweight material that can be used as construction material and carrier for bacteria. In a study conducted by Wang et al. [82], it was discovered that DE particles had pore sizes of 0.1–0.5um. The smaller pore sizes of DE present a challenge in terms of loading capacity for bacteria cells. Due to the bacteria cells being approximately 6 to 10 times bigger than the DE pores, the loading capacity is expected to reduce, similar challenges were faced by Han et al. [29] while using EC. Hence, according to Wang et al. [82], only crack widths of 0.17 mm were able to be healed. A possible explanation for the small amount of healing observed in the latter experimental study, could be attributed to the bacteria not having enough space to grow and multiply when in contact with favourable conditions. Orozco and Urbino [53] studied the use of DE and LWA, to immobilise B. Cibi. They found that the addition of DE led to a 0.22 % increase in compressive strength, indicating minimal impact on the original strength of the specimen. Which were also confirmed by Ersan et al. (2015) which saw similar compressive strength to the reference specimens. Thus, suggesting DE doesn't impact the mechanical properties of the mortar specimens.

Gel like inorganic materials represents potential good carriers for bacteria, such as calcium alginate[27]and silica gel[72]. The use of silica gel as a carrier showed promising results of 100 % decrease in water permeability in 10 days of cracking[72]. The samples treated with bacteria immobilized within silica gel were able to achieve similar water permeability levels attained using epoxy. However, the study didn't evaluate the possible adverse effect on the mechanical properties caused by the presence of the inorganic carrier. This latter aspect should be included in future study in addition to durability and self-healing performance.

Construction Demolition Waste (CDW) amounts for 30–35 % of solid waste produced globally[23,25]. The use of waste materials as potential carrier provides an opportunity to protect the environment and preserve natural resources moving towards more sustainable constructions. Khushnood et al. [47], utilized recycled concrete aggregate from CDW as a carrier material for non-ureolytic bacteria, and they observed complete healing of 0.70 mm cracks and regain of 73 % compressive strength after 28 days healing. Han et al. [28] adopted recycled concrete aggregate and observed decreasing water absorption up to 3.3 % after bacterial immobilization. Xu et al. ((2024) also studied the use of recycled concrete aggregate finding that after 28 days 0.30–0.40 mm were able to be sealed with the material recovering upto 74.4 % water tightness. Wang et al. [84] was able to provide laboratory evidence of successful immobilization of spores using bacterial viability test, additionally, finding specimens were able to regain 99.2 % of its original water tightness. It is evident that available studies utilizing recycled aggregate focus on strength regain, permeability and crack width. Furthermore, limited studies looked at the nanostructure bonding between recycled CDW, $CaCO_3$ and cementitious matrix. This knowledge is significant as improving the bonding between CDW, healed product, and cementitious matrix will help in comprehending the factors influencing the varying mechanical properties. Additionally, X. Wang et al. [84] considered leakage and highlited 12.3 % of bacteria spores leaked out of the recycled aggregate, which they have considered to use coating materials prevent the leaking. This issue has been discussed extensively by previous workers [64,97,58]. A possible alternative to prevent leakage relies on the utilisation of compatible coating material. Macro, micro and nano-scale determinations can be used to develop a holistic understanding of the benefits of CDW.

The use of nanoparticles in construction materials could provide many desirable benefits, including the improving of strength in concrete due to a more densely packed microstructure. Shaheen et al. [63] investigated the healing ability of concrete with the addition of nano/microparticles as carriers for the bacteria. They were able to observe that at 28 days pre-cracking the specimen regained 46 % of its compressive strength using iron nano/microparticles, compared to the use of bentonite nano/microparticles, which was associated to only a 31 % regain of the compressive strength. Khaliq and Ehsan [45] studied the use of graphite nanoparticles which was able to achieve complete healing of crack widths of up to 0.38 mm, whereas [63] were able to observe considerably greater healing with iron oxide nano/micro particles (INMP) at 1.20 mm (Fig. 5). The use of INMP showed to be associated to the maximum crack width healed. The small sizing of the carrier also enables uniform distribution of bacteria. In addition to imparting self-healing benefits, the use of carrier materials enhances the mechanical strength of cementitious materials[45,50,62], as the smaller sized particles can act as filler materials to improve compaction of the matrix.

Nanoparticles were responsible for a 9–50 % improvement of compressive strength. Similar results were reported at 50 % increase with the addition of 0.3 % weight percentage of cement used[50]. Given the promising results obtained using GNP, INMP and BNMP nanoparticles as carriers in terms of self-healing and mechanical properties, scholars should explore cheaper and renewable source to produce effective bacterial carrier materials (e.g., nanocellulose).

Fig. 6 shows the measurement of crack widths healed by researchers to visual self-healing, using crack width to monitor the healing process by taking images at 14 and 28 days of healing [35,45,63,86,91]. Table 5 shows various other techniques commonly used to measure self-healing, including regain strength [56,57,63,84,93,92,92,93]which identify whether the healed specimens are able to regain its original strength, indicating successful self-healing. Specimen may seal the crack mouth without recovery of strength, following the principle of self-sealing [68,71]. Another popular technique in literature is water permeability test[32]; J. [76]; Wu, et al., 2019; Xu et al., 2024), which tests the material durability after healing of cracks. The ability of the cracked material to transport water indicates the efficiency of self-healing over the healing period. Regaining water tightness prevents harmful substances from entering and deteriorating the cementitious matrix and reinforcements.

3. Immobilization methods

In order to understand the behaviour of bacteria in a self-healing concrete system, the methods available to introduce the bacteria into carriers require to be systematically studied. Immobilization is needed to prevent free movement of the bacteria and project them. Hence this section will focus on porous materials, and the bonding between bacteria and the carrier material. Previous studies have emphasised the importance of protecting bacteria and a variety of immobilization techniques have been attempted. The most common immobilization techniques are summarised in Fig. 6, namely i) Adsorption Treatment Method (ATM)[4,45,64,82], ii) Micro-Encapsulation Method (MEM) [96,27,35,79], iii) Vacuum Impregnation Method (VIM)[36,48,86,97] (See Fig. 7).

Table 6 provides a summary of immobilization methods, performance levels and reference studies. As visible in Table 6, the use of MEM was able to heal crack widths of up to 0.97 mm after being pre-cracked after 28 days. The results confirm that utilising MEM facilitates the ability to (i) transfer high density of bacteria, (ii) protect bacteria from the concrete environment and (iii) make them available to heal cracks as and when they occur. However, out of the three methods, MEM is the most complex and expensive[14], due to the adoption of polymeric materials (such as alginate, polyurethane and melamine) and cross-linking agents.

ATM and VIM methods are similar as they both utilize the mechanisms of adsorption and absorption. ATM method is simple, easy and cheap as the carrier materials are left to soak in bacterial solution and then nutrients. After each soaking period, the porous carriers with bacteria are dried using an oven[45,48,86,100]. VIM method uses vacuum conditions and pressure to push the bacteria deep into the pores of the porous carrier materials[58]. Different approaches in transporting the nutrients into the concrete to be available to the bacteria have been tested, such as (i) to incorporate the bacteria and then the nutrients in carrier materials (Ersan et al., 2015), (ii) to add the nutrients and then incorporate the bacteria solution in carrier materials[86]or (iii) to use a bacteria solution and directly add the nutrients in the concrete mix[48]. The direct application of nutrients is often associated with a decrease in mechanical properties and it adversely affects the concrete setting time. As nutrients are crucial components for self-healing cementitious materials, the way in which they are incorporated are important. According to a lab campaign conducted by Jonkers (2010) that the use calcium lactate precursor led to an improvement in compressive strength. In contrast, Paine (2016) found that increasing the concentration of calcium lactate beyond 1 % in mortar specimens caused a decrease in compressive strength, with a similar trend observed by[70,73]).

Additionally, not only do calcium precursors influence materials properties, but nutrients such as glucose and yeast extract also impact

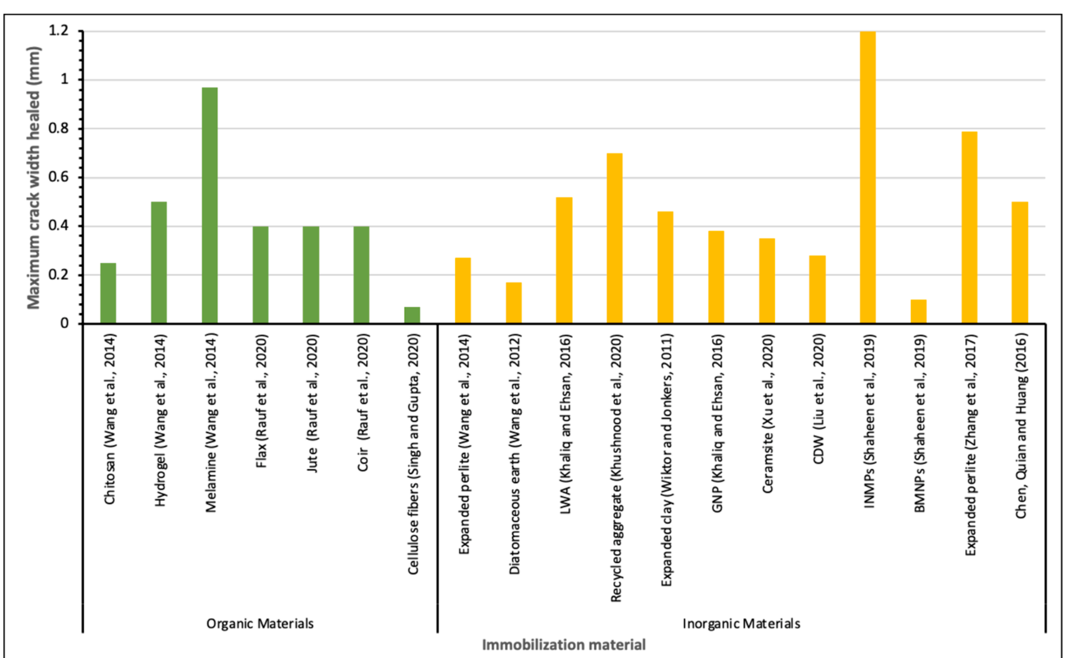

Fig. 6. Summary of maximum crack width healed from literature using various organic and inorganic materials as carriers for bacteria.

Table 4
Summary of various inorganic carrier materials used in literature and the influence on performance of cementitious materials.

Immobilized material	Performance	Reference
EP	• 68.18 % decrease in water surface absorption within 120 min	[1]
EP	• Ability to healing crack widths up to 0.27 mm in 56 days of healing.	[79]
EC	• Maximum crack width healed of 0.46 mm after 100 days healing	[86]
DE	• Slight decrease in compressive strength of 1–3 %.	[22]
Metakaolin	• 5 % increase in compressive strength	
EC	• Slight decrease in compressive strength of 1–3 %	
Zeolite	• Significant decrease in compressive strength of 20.82 %.	
DE	• Ability to heal a maximum crack width of 0.17 mm. • Able to maintain ureolytic activity in pH 12.5	[82]
LWA	• Ability to heal a maximum crack width of 0.52 mm. • 12 % increase in compressive strength compared to control sample	[45]
RA	• Ability to heal a maximum crack width of 0.70 mm. • 3 % decrease in compressive strength when RA with bacteria were introduced • 33.3 % increase in split tensile strength • Regained 73 % compressive strength	[47]
GNP	• Ability to heal a maximum crack width of 0.38 mm. • 9.80 % increase in compressive strength compared to control sample	[45]
Ceramsite	• Ability to heal a maximum crack width of 0.45 mm. • 19 % regain in flexural strength • 17 % regain in modulus strength	[91]
CDW	• Ability to heal a maximum crack width of 0.28 mm. • Water absorption capability 3.83 %. • Healing ratio of 85 %	[48]
INMPs	• Regained 46 % compressive strength after pre-cracking 28 days. • Increase in split tensile test of 7.5 % • Ability to heal a maximum crack width of 1.20 mm	[63]
BNMPs	• Regained 31 % compressive strength after pre-cracking 28 days. • Decrease in split tensile test of 5 % • Ability to heal a maximum crack width of 0.10 mm	[63]
Iron oxide nanoparticles	• Increase in compressive strength of 15 % compared to the control sample.	[62]

cementitious properties. Williams et al., [87] studied the use of glucose in cementitious materials and found even at 1 %, it caused a retardation effect. Similar results were found by [69], in which 1 % delaying the hardening of cementitious materials. This is due to the sugar molecules being effective retardants, solubilising calcium hydroxide and other hydration products, which influence the degree of hydration[69]. Another popular nutrient used in literature is yeast extract (YE), in which both Chen et al., [11] and Jonkers et al., [40] observed a decrease in compressive strength when adding YE, with Ersan et al., (2015) finding an increase in setting time with just 1 % YE. Furthermore, Erşan et al., [22] found that the addition of urea and YE to mortar specimens caused a decrease in compressive strength of up to 15.87 %. Joshi et al., [41] also observed a 15.50 % decrease in compressive strength when urea and calcium chloride were directly added. Researchers found that the addition of nutrients like YE, urea and calcium precursors led to an increase in final setting time [22]; H. [40,41]. This suggests that, similar to glucose, organic nutrients such as yeast extract and urea cause a

Table 5
Techniques to evaluate self-healing.

Techniques	Description	References
Visual inspection of cracks	Observation of cracks under a light microscope over the healing period	(Ahmad et al., 2024; [1,48,57,59]; X. [84]
Water permeability	Observation of material regaining water tightness after healing	(Ahmad et al., 2024; [36,59]; X. [84]
UPV	Non-destructive test which measures changes in concrete structure between cracks and healed specimens	[57,64,92,93,94]
SEM and EDS	Observation of the morphology of precipitate formed and identification of elements present	(Ahmad et al., 2024; Xu et al., 2024; H. [40,47,59]; J. [78]; X. [84]; Wu, Hu, et al., 2019)t al., 2024, [59], Ahmad et al., 2024, [84]
Regain mechanical properties	Measuring the effectiveness of the bonding between the healed product and cementitious matrix	[35,56,57,91,92,93,94]

retardation effect on the hydration within cementitious materials. Williams et al., [87] observed that the addition of urea and yeast extract extended the acceleratory period from 2 h (neat) to 10 h. Therefore, adding nutrients directly into cementitious materials will cause significant repercussions in terms of strength development, as glucose, yeast extract and calcium precursors (i.e., calcium lactate and calcium acetate) are popular choice for nutrients for self-healing agents. Hence, this further stresses the importance of effective immobilisation not just for the bacteria, but also for the nutrients.

The decrease in strength of cementitious materials can also be attributed to the type of bacteria being immobilised, as noted by Mohamed et al., [51]. Erşan et al., [22] found that using carriers such as metakaolin and zeolite in mortar resulted in a 16.67 % decrease in setting times for metakaolin and similar setting times for zeolite compared to control specimens. Additionally, the direct application of bacteria (B. Sphaericus) alone only increased setting time by 20 %. However, when immobilised bacteria and nutrients into metakaolin and zeolite, a significant decrease in compressive strength of 66.55 % and 16.38 % respectively was observed along with an increase in final setting time to 510 min and 530 min. This suggests that the nutrients and bacteria leaked out of pores of these carrier materials, causing a delay in setting time due to the impact of these organic materials on hydration. According to Khushnood et al. [47], the ATM can decrease the absorption of the optimal amount of bacteria suspension during a 24-hour period. The use of Vacuum Impregnation Method is seen to be able to achieve a higher absorption capacity[2,97]. Also, the use of a vacuum can increase the absorption rate (e.g., by 12 % in 30 min)[2]. Considering the studies mentioned in this section, it is clear that a major concern surrounding immobilization in porous materials is represented by the retention of bacteria (Gupta and Singh, 2020; [58]and nutrients. Bacteria leakage is a major challenge and it is caused by weak bonds between the bacteria cells and carrier materials, such as van der Waals forces and hydrophobic interactions[66]. As well as the issues surrounding nutrient leakage causing significant problems to the properties of the cementitious materials. Thus the main techniques used for bacteria and nutrients retention are discussed in detail in the following sections.

3.1. Incorporation of bacteria

Implementation of bacteria into cementitious materials is one of the most important factors as this would allow for more effective healing to take place. This section will discuss the incorporation of bacteria into

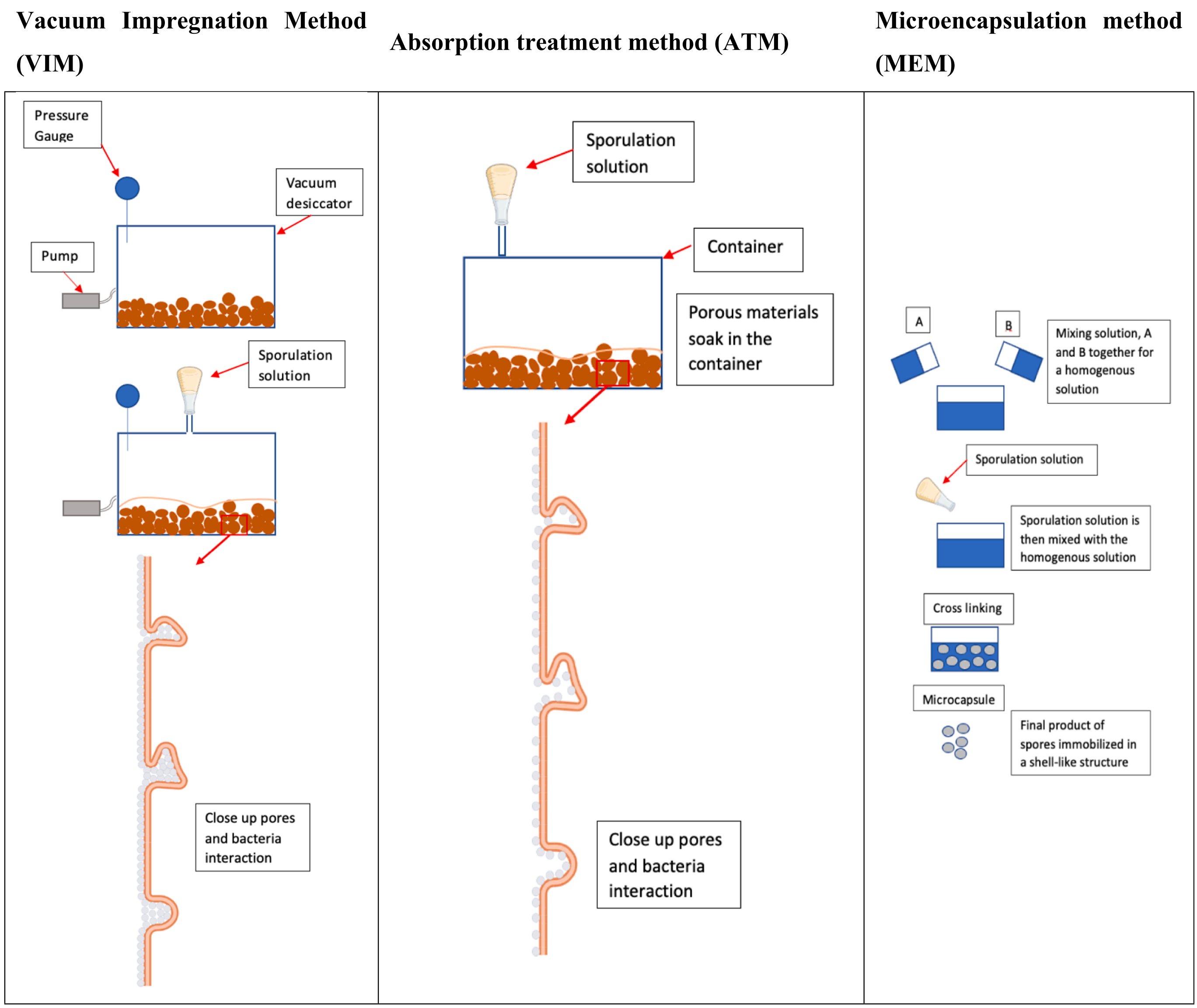

Fig. 7. Schematic diagrams of the different methods used in literature to immobilize bacteria for self-healing purposes.

Table 6
Summary of methods used to immobilize bacteria into carrier materials.

Immobilization method	Carrier material	Immobilization technique	Performance	Reference
	LWA	Adsorption treatment method	• Reduction in water absorption of 10 %	[4]
Absorption and Adsorption	EC	Vacuum impregnation method	• 0.46 mm crack width completely healed after 100-day healing.	[86]
	RA	Vacuum impregnation method	• 0.70 mm crack width healed completely after 28 days • Recovered 73 % of compressive strength after 28 days healing	[47]
	LWA	Adsorption treatment method	• 0.51 mm crack width healed completely after 28 days	[45]
	EP	Vacuum impregnation method	• 0.79 mm crack width healed completely after 28 days	[97]
	DE	Adsorption treatment method	• 0.17 mm crack width completely healed after 40-day healing.	[82]
	EP	Vacuum impregnation method	• Able to heal crack width of 0.81 mm. • Improved water permeability by preventing seepage.	[36]
	Cellulose fibres	Adsorption treatment method	• After 14-days pre-cracking specimens showed 12.04 % self-healing.	[64]
	Natural fibres	Adsorption treatment method	• 0.50 mm crack width completely healed after 28-day healing.	[57]
	RA	Vacuum impregnation method	• 0.28 mm crack width completely healed after 28-day healing.	[48]
Encapsulation	Melamine	Micro-encapsulation	• 0.97 mm crack width completely healed after 56-day healing	[79]
	Sodium Alginate	Micro-encapsulation	• Regained 46 % of ultimate load when compared to control specimen	[35]
	Calcium alginate	Micro-encapsulation	• Healing ratio of approximately 67 %	[27]
	Hydrogel	Micro-encapsulation	• Maximum crack width healed 0.50 mm • Average decrease of water permeability of 68 %.	[76]

matrix through various transportation technologies. A primary concern in the industry revolves around the application of bacteria, in particular the options of incorporating the bacteria directly or indirectly through the use of immobilization methods and materials as seen in Table 7. According to the technical literature, the way bacteria are introduced not only influences the mechanical properties but also the self-healing capabilities. Researchers explored two main methods: i) direct application, which can be separated into mixing with the cement paste [39,60,24,38,40]or external direct application as a surface treatment [16,52,88,90]and ii) indirect application, involving the use of immobilization materials. This latter method involves the bacteria being placed in a carrier material, which is mixed with the cement paste [45,47,79,86,97]. According to Jonkers et al. [40], directly introducing the bacteria into the paste leads to 10 % decrease in compressive strength after 28 days. These findings suggest that the bacteria viability decreases due to the harsh conditions found in the cementitious materials. Similar outcomes were observed by other researchers[82,76]. They exposed bacteria to high alkaline pHs (pH>9) and observed a drastic decrease in bacterial activity (i.e., 94.12 % decrease in ureolytic activity of bacteria when exposed to 12.5 pH formed from cement slurry). Such results highlight a concerning problem related to this kind of bacteria application. The relationship between bacterial viability and pH must be studied further to decrease adverse effects and maximize the healing performance.

pH is not the only factor causing cells to lose viability and affecting the production of $CaCO_3$. Production of $CaCO_3$ can be influenced by mechanical stresses due to mixing of the cementitious material resulting in crushing of the bacteria. Aging of the cementitious materials represents another important factor to be considered when evaluating bacterial viability [80]. Indeed, bacterium cells range from 1-3 mm[80], whereas pores in cementitious matrix is estimated at 0.5 mm. As the cementitious material ages, the microstructure of the materials becomes denser and more compact, meaning pores could possibly get smaller [76,63,97]. Jonkers et al. [40] found that pores in young specimens (3 and 7 days) were larger (size range of 0.1–1 mm) and smaller in mature specimens (size of 0.01–0.1 mm after 28 days). This finding shows that as the specimens age the microstructure will exert stress on the exposed bacteria cells and lead to bacteria cells to be crushed. Similar results were obtained by other researchers [45,47,63]. Those studies agreed that a decrease in healing potential, the width of crack that was able to be healed is directly caused by specimens aging. An argument made by Wang et al. [76] supported this, suggesting that the reduction in pore size resulting from microstructure development due to aging exerts uniaxial loading on bacteria cells. Consequently, the cells become crushed, rendering them ineffective. The use of indirect application is seen to improve healing capabilities as seen according to Khaliq and Ehsan [45] when direct application of bacteria was only able to heal 0.15 mm, whereas immobilized in LWA it was able to significantly improve crack width healed by 246.67 %. Similar trends to this were also seen by Shaheen et al. [63] and Khushnood et al. [47]. According to Wang et al. [76], a likely explanation for the survival of the bacteria in pores of porous materials was due to the creation of localised pH being lower than the pH)found in cementitious materials pore, increasing the number of bacteria that survives, hence, enabling it to take part in healing mechanisms. The carrier materials also provide concrete protection from the mechanical stresses as they become part of the cementitious matrix. However, the selection of immobilizing material is important, as they should provide enough support not to crack during mixing and provide enough exposure for the bacteria to interact with the external environment once cracks appear.

Table 7
Summary of literature of the various applications of bacteria in self-healing material.

Type of application	Immobilizing material	Bacteria	Performance	Reference
Direct	N/A	B. Cohnii	• Reduction in spore viability of 94.12 % after 22 days of curing. • Decrease in compressive strength of up to10%.	[40]
Direct	N/A	B. Subtilis	• Able to heal a crack width of 0.15 mm	[45]
Immobilized	LWA	B. Subtilis	• Able to heal crack width of 0.52 mm	[45]
Direct	N/A	B. Subtilis	• Improved compressive strength by 8 %. • Healed crack width of 0.44 mm. • Able to regain 32 % compressive strength	[63]
Immobilized	IONP	B. Subtilis	• Improved compressive strength by 21 %. • Healed crack width of 1.20 mm. • Able to regain 46 % compressive strength	[63]

3.2. Drying

Oven drying is commonly used for the carrier material in the temperature range of 30 °C – 45C (e.g. [36,48,86,97,100]. The bacteria genus reported for self-healing *Bacillus* is a mesophile bacterium with an optimum temperature range of 20 °C – 45 °C[10]. This is problematic because the temperature used to dry carrier materials might lead to an increase in metabolic activity of the bacteria spores, initiating the germination process. As a result, drying process could lead to less viable bacteria for the healing of cracks due to the fact that crystallization has already taken place within the porous carrier. Alternative drying of carrier materials is aimed at ensuring that the healing process takes place inside the concrete once cracks propagate. Such technology employs freeze-drying technique able to develop the process at temperatures as low as −48 °C, while keeping the bacteria viable in the carrier material[42]. Bauer et al. [7], employed freeze-dry processes but proposed a method of Controlled Low Temperature Vacuum Dehydration (CLTV), which produced survival rates of bacteria comparable to freeze-drying. Pungrasmi et al., [54] employed various drying techniques such as spray drying and freeze drying. In their study, which aimed at drying sodium alginate microcapsule with bacteria spores inside, they found that freeze-drying retained 100 % survival of bacteria spores compared to 79.9 % achieved with spray drying. This was due to the high temperature associated with spray drying, which in this case L. Bulgarius ribosome melt at 60C, leading to DNA denaturation [54]and disruption of the binary fission process. Although these methods are not primarily intended for self-healing, they enable researchers in the field to focus on effectively controlling bacterial activity during the immobilization process. It is important to highlight that it is difficult to control the temperature of the carrier material once within the concrete matrix. Therefore, an option could be to use coating materials around the carrier materials to restrict oxygen availability for the bacteria, thereby preventing the activation of spores. Overall, further research is required in this area. According to the technical literature, how bacteria react at specific temperature ranges has been clarified. It is not yet clear how this affects self-healing performance and whether using cooler temperatures for drying could help preserve bacteria, making them readily available, when cracks develop in the matrix.

3.3. Coating

Immobilizing bacteria into porous carriers is not sufficient in optimising the healing process. Singh and Gupta [64] attributed the poor self-healing performance of cellulosic fibres to be a result of bacteria retention. Hence, they were successful in demonstrating that if the bacteria are unable to remain within the porous carrier material during the mixing step, poor healing performance could result. Additionally, an experimental investigation using Diatomaceous Earth (DE) showed that as the pH increased in the environment in which the DE carrier was found, the activity of ureolytic bacteria decreased significantly by Wang et al. [82]. This decline in ureolytic activity can be attributed to the open pores found within the DE carrier material, which provide a point of entry for the high pH environment. As previously mentioned, Jonkers et al. [40] argued that bacteria required protection from high alkaline environments, which can impact the bacterial viability. To this scope, coating of carrier materials is employed [1,36,97,58]. The coating material is applied on the surface of the carrier material to prevent the leakage of the contents inside the carrier and prevent initial mixing environment from reducing the viability of the bacteria. Various coating materials have been proposed, such as geopolymer (Lin, et al. 2021; [36,97], Portland cement[36], MKPC[36], Sodium silicate[58]and Sodium alginate[58]. Zhang et al. [97] utilised sodium silicate and metakaolin as coating materials for EP particles, resulting in a 225 % decrease in water absorption compared to non-coated EP particles. Yan et al. [94] coated EP with potassium dihydrogen phosphate and magnesium oxide sprayed on the surface, which was able to heal crack widths of 0.60 mm and aid in regaining compressive and flexural strength of 91.47 and 97.13 %, respectively. This suggested the coating material was able to bond well with the cement matrix, calcium carbonate and EP to nearly achieve its original mechanical properties. [30]observed the use of EC coated with styrene –acrylic and found a more rapid decrease in bacteria density in the coated EC compared to uncoated. This decrease in bacteria density may be attributed to the immobilisation process of using ethanol and UV light to kill bacteria on the surface, as the open pores of EC could subjected parts of the immobilised bacteria to these conditions. The researcher also found that uncoated EC and coated EC achieved self-healing rates of 70 % and 75 % respectively, suggesting a slight improvement in healing due to the coating protecting the bacteria from external environment.

Another laboratory campaign conducted by Risdanareni et al. [58] demonstrated promising results, reporting a 24.05 % decrease in bacteria leakage when using sodium alginate coating in comparison to uncoated LWA. Xu et al., (2024) studied the use of sodium silicate coating on recycled concrete aggregate (RCA), with double coating to prevent leakage. They observed that a 40 % replacement of RCA led to a complete healing of crack widths up to 0.63 mm indicating the bacteria were effectively retained within the pores. Both Zhang et al. [97] and Jiang et al. [36] have been successful in keeping the bacteria viable, while simultaneously minimizing leakages, using low alkalinity coating materials versus high alkalinity materials. This suggests that the use of low alkaline coating material will enable the bacteria to remain viable due to the local environment being less alkaline. Furthermore, when selecting a coating material, suitability is highly dependent on the compatibility of the coating material with both bacteria and the concrete matrix. Previous studies focused on coating materials have not addressed how the coating material affects the mechanical properties of concrete and whether the coating material affects the bond between the carrier material and the matrix.

4. Conclusions

This review has studied the various materials used for immobilization of bacteria with the materials being categorised into two categories of organic and inorganic materials. The various techniques used to immobilize bacteria from impregnating materials to drying and coating the materials were also explored. The main conclusions to be drawn from the review are as followed:

1) Organic and inorganic materials have been commonly used in the immobilization of bacteria for self-healing application, with both proving to be effective in protecting bacteria and supporting bacteria viability, though inorganic nanomaterials exhibited superior properties and self-heling ability in cementitious materials, healing wider cracks width compared to organic and other inorganic materials.
2) Material selection should strongly consider bonding between carrier material, healing product and cementitious matrix, to provide a self-heling system which amplifies healing of cracks but also maintains the mechanical properties of the cementitious material.
3) Further research should prioritize the exploration of carrier materials derived from natural renewable resources, specifically nanocellulose particles and waste materials to provide a more sustainable carrier material.
4) The selection of immobilization methods significantly affected the loading capabilities of bacteria into carrier materials, Additionally, the immobilisation of nutrients is also important to prevent any negative impact on the properties of cementitious material, VIM demonstrated superior results to ATM and offered a less complex and cost-effective approach than MEM.
5) Additional research is required to investigate drying methods for various carriers to understand how these methods impact the carrier materials. Selecting suitable drying methods will effectively prevent the premature activation of bacterial spores during the drying of immobilization materials once they are impregnated. Exploring low temperature drying methods may offer a potential solution to addressing the issue of premature activation of spores.
6) Coating materials not only offered protection to the bacteria embedded within pores but also prevented the leakage of bacteria and has the potential to improve the bonding between the cementitious material and immobilization material. However, further research is needed to find an environmentally friendly option.

Overall, the selection of immobilization materials and methods are crucial for creating a successful self-healing system, which provides effective healing of cracks and maintains the mechanical properties of the cementitious materials. The ideal carrier should possess biocompatibility, compatibility with cementitious matrix, good mechanical properties, high loading capacity, low cost, environmentally friendly and be renewable. As a result, the materials able to meet the criteria stated have potential to be an effective carrier material for self-healing technology and achieve a balance between self-healing and mechanical properties of building materials.

CRediT authorship contribution statement

Abdulahi Mohamed: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Mizi Fan:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Elisa Bertolesi:** Conceptualization, Supervision. **Hanyuan Chen:** Data curation, Resources. **Ziyan Fu:** Data curation, Formal analysis, Resources. **Terry Roberts:** Data curation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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