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Review

Application of Carrier Materials in Self-Healing Cement-Based Materials Based on Microbial-Induced Mineralization

Chunhua Feng , Xudong Zong, Buwen Cui, Hui Guo, Wenyan Zhang and Jianping Zhu *

School of Materials Science and Engineering, Henan Polytechnic University, Jiaozuo 454000, China; fengchunhua@hpu.edu.cn (C.F.); 212006010009@home.hpu.edu.cn (X.Z.); 211906020032@home.hpu.edu.cn (B.C.); guohui821@hpu.edu.cn (H.G.); zhangwy@hpu.edu.cn (W.Z.)
* Correspondence: jianpingzhu@hpu.edu.cn; Tel.: +86-135-6917-2672

Abstract: Microbially induced calcium carbonate precipitation (MICP) technology has attracted widespread research attention owing to its application in crack healing for cement-based materials in an intelligent and environmentally friendly manner. However, the high internal alkalinity, low nutrient content, and dense structure of cement-based materials have restricted its application in self-healing cement-based materials. Various carrier materials have been widely used for the immobilization of microorganisms in recent years. Carrier materials have significantly increased the ability of microorganisms to withstand extreme conditions (high temperature, high alkali, etc.) and have provided new ideas for the compatibility of microorganisms with cement-based materials. In this study, the basic principles of microbial self-healing technology in cement-based materials and microbial immobilization methods and the influencing factors are introduced, followed by a review of the research progress and application effects of different types of carrier materials, such as aggregate, low-alkali cementitious materials, organic materials, and microcapsules. Finally, the current problems and promising development directions of microbial carrier materials are summarized to provide useful references for the future development of microbial carriers and self-healing cement-based materials.

Keywords: cement-based materials; microbially induced mineralization; self-healing; carrier material; microbial immobilization

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Citation: Feng, C.; Zong, X.; Cui, B.; Guo, H.; Zhang, W.; Zhu, J. Application of Carrier Materials in Self-Healing Cement-Based Materials Based on Microbial-Induced Mineralization. *Crystals* **2022**, *12*, 797. https://doi.org/10.3390/cryst12060797

Academic Editor: Carlos Rodriguez-Navarro

Received: 30 April 2022
Accepted: 31 May 2022
Published: 3 June 2022

1. Introduction

The cracking of cement-based materials in service affects the appearance of buildings and reduces the load-bearing capacity and durability of the building structure, a problem facing the construction industry. Microbial metabolism induces microbially induced calcium carbonate precipitation (MICP), a process that can proceed spontaneously under suitable environmental conditions and is environmentally friendly [1]. With the massive application of bioengineering in the construction industry, although numerous scholars have applied MICP technology to repair cracks in cement-based materials, the high alkaline environment and dense structure inside cement-based materials restrict its application and development [2]. Although the addition of mineral admixtures, such as silica fume and fly ash, to cement-based materials can improve the high alkaline environment and relieve the environmental pressure for microbial survival, the active components in these mineral admixtures react with the cement hydration product calcium hydroxide to produce hydrated calcium silicate or hydrated calcium aluminate. The pH of the pore solution is effectively lowered [3,4], the change in pH can accelerate the rate of corrosion of reinforcing steel and endanger structural safety. Meanwhile, the use of silica fume increases the self-shrinkage of cement-based materials, and very fine silica fume particles refine the internal pore structure of the cement-based materials [5], limiting the microbial living space to some extent.

Currently, the use of carriers to immobilize microorganisms is an effective method for enhancing the resistance of microbial environments, and various carrier materials have been adopted, such as diatomaceous earth (DE), expanded perlite (EP), expanded clay (EC), granular activated carbon (GAC), polyurethane (PU) foam, rubber, hydrogel, melamine [6–10]. The carriers provide sufficient microbial living space and help avoid direct contact between microorganisms and the high alkaline environment inside cement-based materials. In addition, carriers can control the nutrients around the microorganisms, forming a nutrient environment that favors microbial growth and reproduction. Therefore, microorganisms protected by carriers can perform mineralization deposition effectively, inducing improved crack healing.

In previous studies on self-healing cement-based materials based on microbial-induced mineralization, the focus was mainly on the self-healing effect of cracks, and a systematic and comprehensive study of the carrier was lacking. This paper introduces the basic principles of microbial self-healing technology, microbial immobilization methods, and influencing factors, reviews the applications and characteristics of different types of carrier materials, such as aggregates, low-alkali cementitious materials, organic materials, and microcapsules, based on relevant research progress, and highlights the future development direction of microbial carrier materials.

2. Basic Principles of Microbial Self-Healing Technology

Biomineralization is the process of converting ions in the environment into inorganic minerals through the participation and regulation of the organisms' cells [11]. Depending on the degree of microbial control of mineralization, microbial mineralization deposition is usually classified into two types: microbially controlled mineralization and microbially induced mineralization [12,13]. In microbially controlled mineralization, microorganisms control the nucleation and growth processes of minerals to a significant extent, and the minerals formed have different characteristics with different microbial species. However, for microbially induced mineralization, mineral formation depends on the combination of the surrounding environment and microorganisms [14]. Studies have shown that the microbially induced mineralization process is easier to control and more suitable than microbially controlled mineralization is for general application in engineering. Hence, the former is currently widely used in the self-healing of cracks in cement-based materials.

The process of microbially induced mineralization deposition can be summarized as microorganisms producing ${CO_3}^{2-}$ through biochemical processes, such as urea–urease reaction, nitrate reduction, and sulfate reduction, and continuously chelating Ca^{2+} in the environment through microbial cell membranes to finally mineralize and deposit $CaCO_3$ crystals with cementation ability [15–17].

Qian et al. [18] studied the process of calcium carbonate deposition in alkali-tolerant *Bacillus* spp. from four aspects: mineralization products, pH, O_2, and substrate, respectively. They found that the mineralization process requires O_2 participation, and the substrate and nutrients provide Ca^{2+} and ${CO_3}^{2-}$ required for mineralization, which eventually produces calcite-type $CaCO_3$ through various metabolic transformations of bacteria. Based on the above findings, they briefly summarized the calcium carbonate deposition process in the following three steps.

$$\text{Sources} + O_2 \xrightarrow{\text{Bacteria respiratory metabolism}} CO_2 + H_2O \tag{1}$$

$$CO_2 + OH^- \rightarrow HCO_3^- \tag{2}$$

$$\text{Cell} - Ca^{2+} + HCO_3^- + OH^- \rightarrow \text{Cell} - CaCO_3 \downarrow + H_2O \tag{3}$$

Zhong et al. [19] highlighted that calcium carbonate precipitation induced by microorganisms has some cementation ability. The mineralization products are mainly calcite-type calcium carbonate, which comprises many fine grains with irregular shapes and different orientations, as well as rough surfaces and large specific surface areas, easily adsorbing and aggregating loose particles from outside, thereby exhibiting strong cementation ability [20–22].

This group summarized the principles and process of MICP technology for crack healing in cement-based materials (Figure 1). Microorganisms are placed inside cement-based materials, and when cracks are created, external moisture and oxygen infiltrate and activate dormant microorganisms and participate in microbial respiratory metabolism, microorganisms produce CO_2 and continuously chelate Ca^{2+} in the environment with themselves as nucleation sites [23,24]. Calcium carbonate is continuously produced in cracks via microorganism induction and fills and bonds the cracks until the purpose of crack healing is realized.

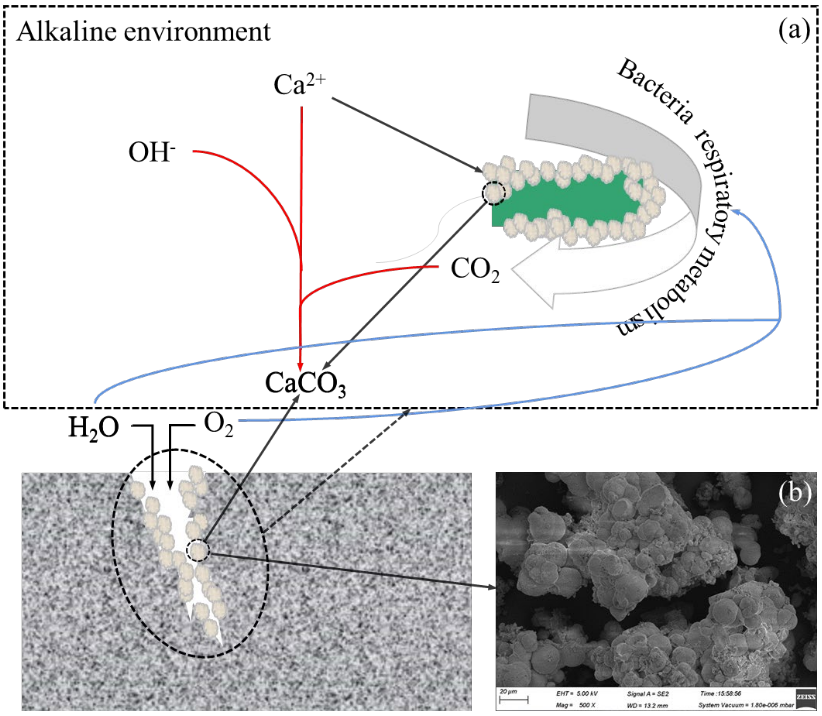

Figure 1. The principles and process of MICP technology for crack healing: (**a**) Microbially induced $CaCO_3$ precipitation in cement-based material cracks; (**b**) Morphology of calcite formed by microbial induction.

3. Microbial Immobilization Methods and the Influencing Factors

3.1. Microbial Immobilization Methods

Presently, the main microbial immobilization methods are adsorption, covalent binding, encapsulation, flocculation, and so on. Figure 2 shows a brief diagram of the working principles of several methods.

1. Adsorption, a simple and reversible process, is a commonly used physical method to immobilize microbial cells through interactive forces, including van der Waals forces, hydrogen bonding, and ionic bonding between microorganisms and the carrier surface [25–27]. Microorganisms can easily escape into the surrounding environment due to poor bonding between weak bonds. To improve the carrier's adsorption efficiency, the carrier is generally wrapped with non-water-soluble substances, or adsorbents, which are used to enhance the adsorption effect when the microorganisms are immobilized.
2. Unlike adsorption, the covalent binding method requires the use of a binding agent. The groups on the microbial cell membrane (amino, carboxyl, imidazole, etc.) and the functional groups on the surface of the carrier (amide, ether, etc.) are firmly connected by covalent bonds with the action of the binding agent, which is an irreversible immobilization treatment method [28–31]. Binding agents are usually biologically harmful and can adversely affect a cell's activity. Therefore, this method is rarely used for the immobilization of whole cells, mainly for the immobilization of enzymes [27].

3. Encapsulation is also an irreversible method of immobilization. Microorganisms are encapsulated in a restrictive semi-permeable membrane and can only move within the membrane, and the semi-permeable membrane allows nutrients to flow into the membrane, protecting the microorganisms from the internal environment of the cement-based material and maintaining a high level of microbial activity [25,30,32]. The need to accurately control the pore size of the membrane is the key to this technology. When the membrane pore size is oversized, it can cause microbial leakage, and the opposite can affect nutrient flow.
4. Flocculation is divided into natural flocculation and artificial flocculation. Natural flocculation is a method of using certain microorganisms that have the property of self-flocculating, which are combined to form an aggregation. Artificial flocculation refers to the method that reacts to the groups on microbial cell surfaces with cross-linking agents, causing the cells to associate with each other and form a meshwork structure [29,33,34]. Flocculation can increase microorganism concentrations and facilitate the coordination of microbial metabolism. However, natural flocculation consumes time and is easily affected by the external environment. Due to the intense chemical reaction in the cross-linking process, artificial flocculation can cause damage to microorganisms [35,36]. Therefore, flocculation requires improvement.

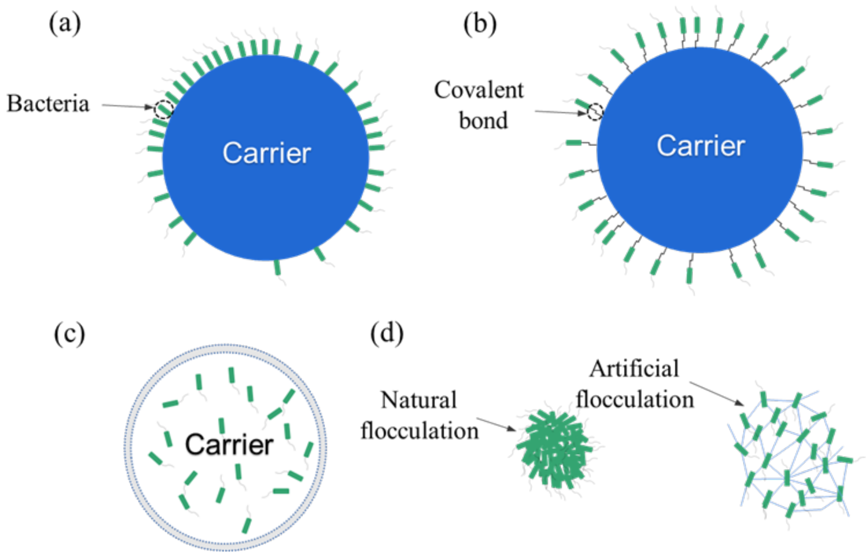

Figure 2. Microbial immobilization methods: (**a**) Adsorption; (**b**) Covalent binding; (**c**) Encapsulation; (**d**) Flocculation.

3.2. Factors Influencing Microbial Immobilization

The coupling of various factors determines the effect of microbial immobilization treatment, and the main influencing factors include carrier performance (e.g., surface roughness, porosity, etc.), environmental factors (e.g., pH, temperature, etc.), and microbial cell status (e.g., age, physiological state, etc.). Table 1 summarizes the influences of different factors on the microbial immobilization process. The rougher the carrier surface, the more developed the pore structure, and the better the attachment and immobilization of microorganisms on its surface. The carrier needs to be non-toxic and harmless, otherwise, the activity of the microorganism will be endangered. In addition, the immobilization process of microorganisms generally takes place in liquids, its solubility needs to be minimal as possible. Changes in environmental factors, such as pH, ion concentration, and temperature, can cause changes in cell membrane potential and affect the electrostatic interaction between the cell membrane and the carrier surface, disadvantaging the adsorption and immobilization of microorganisms. Microbial cell states determine biological activity. The higher the cell's activity, the stronger the ability to resist the negative elements of the immobilization process [27,37–39].

Table 1. Factors affecting the immobilization of microbial cells [27,37–39].

Parameter Type	Parameter	Change	Effects
Carrier performance	Surface roughness	↑	Positive
	Porosity	↑	Positive
	Solubility	↑	Negative
	Biological hazard	↓	Positive
	Hydrophobicity	↑	Negative
	Type of functional groups	-	Neutral
Environmental factors	pH	↑/↓	Negative
	Temperature	↑/↓	Negative
	Flow velocity	↑	Negative
	Ion concentration	↑/↓	Negative
	Cell concentration	↑	Positive
	Binding agent/Adsorbent	-	Positive
Microbial cell	Cells age	↑	Negative
	Physiological state	↑	Positive
	Surface structure	-	Neutral

Note: ↑—increase, ↓—decrease, ↑/↓—too high or low.

4. Types and Applications of Carrier Materials

Excellent biocompatibility, good insolubility in water, high loading capacity, good mass transfer performance, stable structure, long service life, low price, and convenient immobilization should characterize carrier materials for microorganism immobilization [29,40]. Researchers have developed various carrier materials in the field of the self-healing of cracks in cement-based materials, which are mainly classified into four categories: aggregate, low-alkali cementitious materials, organic materials, and microcapsules, according to the nature and morphology of the carrier materials.

4.1. Aggregates

Aggregate-type carrier materials have high mechanical strength and are widely sourced. Common carrier materials include DE, EC, EP, expanded vermiculite (EV), zeolite, and GAC [5–7,41–45], whose microstructures are shown in Figure 3. These carrier materials usually exhibit porous structures, among which DE is a hollow particle with a fishing net-like surface. EC, EP, and GAC have abundant hollow channels on their surfaces. However, EV has a laminar structure with many voids left between the layers. Zeolite has many outwardly projecting plate-like structures in its microscopic morphology. These microscopic features make microorganisms have more adsorption areas and living spaces, which effectively improve the loading efficiency of carrier materials.

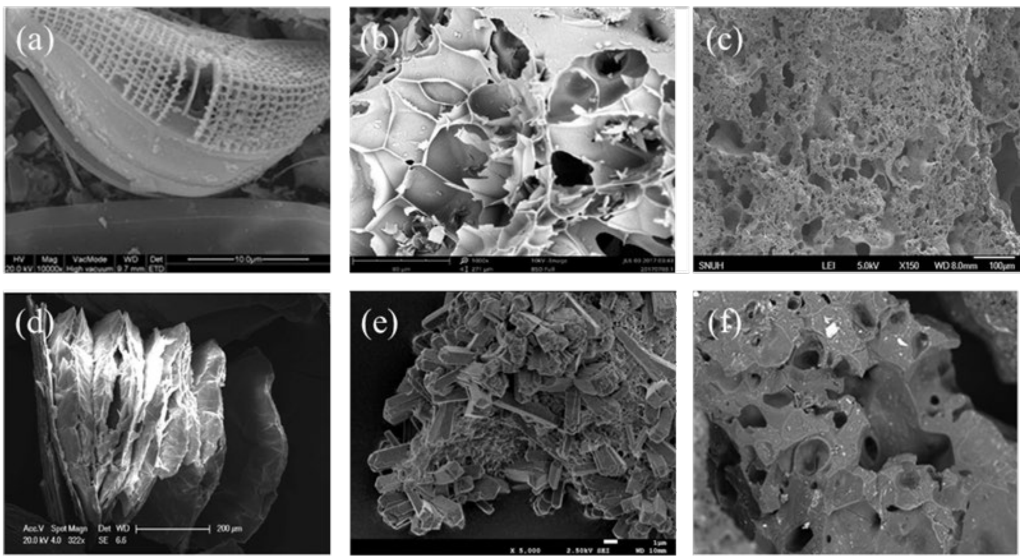

Figure 3. The microstructure of different materials: (**a**) diatomite [46]; (**b**) expanded perlite [42]; (**c**) expanded clay [43]; (**d**) expanded vermiculite [45]; (**e**) zeolite [47]; (**f**) granular activated carbon [48].

Zhang et al. [7] adsorbed microorganisms into EP and EC, respectively, using a vacuum impregnation method. EP and EC have high porosity and high water absorption, once cracks appear in the concrete matrix, the high porosity structure can provide enough oxygen to the immobilized bacteria in the concrete and the high water absorption can expose the bacteria to sufficient water. After specimens with cracks were cured under 20 ± 2 °C and 95% humidity for 28 d, the healing width of concrete cracks mixed with EP particles was 0.79 mm, whereas the healing width of those mixed with EC particles was only 0.45 mm. EP particles had higher bacterial content, lower incorporation, and better crack healing than EC particles. Similarly, using the vacuum impregnation immobilization method, Boon et al. [8] evaluated two carriers, EC and GAC, and showed that the latter had a larger range of crack healing widths and a higher healing limit. Huynh et al. [6] also obtained good crack self-healing using DE-immobilized microorganisms with a maximum crack self-healing width of 1.8 mm.

Bhaskar et al. [44] and Zhan et al. [45] investigated the effects of immobilized microorganisms on the mechanical properties of cement-based materials using zeolite and EV as carrier materials, respectively. The results showed that the microorganisms protected by the carriers remained highly active in a high-pH environment. The mineralized deposited calcium carbonate continuously filled the mortar pores and tiny cracks, and the compressive strength of the specimens containing immobilized microorganisms was significantly increased compared with the control specimens without adding carriers and bacteria at a certain carrier admixture and before cracking.

The particle size of ceramsite (an EC) is generally less than 5 mm, and the temperature influences its porosity. In the range of 400 °C to 800 °C, small pores fuse into large pores, and swelling occurs. When the temperature increases further, the viscosity decreases excessively, causing shrinkage and condensation of sintering products and a reduction in porosity [49]. To load the ceramsite with more microorganisms, XU et al. [50] improved the porosity of the ceramsite by heat treatment and noted that the ceramsite had the highest loading at a heating temperature of 750 °C. In addition, in a study of crack self-healing, they found that, compared with the control group without adding carriers and bacteria, the compressive strength of the bacterial test group protected by ceramsite increased by over 20%, the water absorption rate decreased by about 30%, the healing rate of cracks reached 86%, and the maximum healing width of cracks was 0.3 mm. This is because ceramsite can provide a good living environment for ureolytic bacteria, which can maintain bacterial activity during urea decomposition, and concurrently, bacteria can obtain nutrients in ceramic pellets over time, thereby enhancing the crack healing effect remarkably.

In addition to the aggregate-type carrier materials mentioned above, some researchers have used recycled aggregates (RA) to immobilize bacteria. Natural aggregates and cement-based hydration products in abandoned buildings are the main components of RA, and its surface is rough and angular. The attached irregular old cement mortar accounts for its higher porosity and water absorption than natural aggregates. A large amount of old mortar attached to the RA surface is carbonized for a long time, causing a reduction in the RA surface pH [51–53]. Hence, the RA surface environment favors microbial survival. Liu et al. [51] compared four methods: direct introduction of bacteria, DE-immobilized bacteria, EP-immobilized bacteria, and RA-immobilized bacteria. After the specimens were repaired for 28 days, the crack healing width of the specimens in the RA group exceeded those of the other three, indicating the significance of the porosity and low alkalinity of the RA surface in protecting the bacteria and improving its mineralization deposition.

Aggregate-type carrier materials have high porosity and a specific surface area and immobilize bacteria mainly by adsorption, a process that is simple to operate, low cost, and can maintain high microbial activity. However, there are problems, such as low binding strength between the carrier and the microorganisms, and currently, this problem is mainly solved by using non-water-soluble substances to wrap the carriers or adding adsorbents.

4.2. Low-Alkali Cementitious Materials

Many scholars support sulfo-aluminate cement (SC) due to its high early strength, corrosion resistance, low alkalinity (the pH of the pore solution is about 10.0), high frost and seepage resistance, and good compatibility with silicate cement. By wrapping the spores and nutrients in SC, Zheng et al. [54] made a core-shell microbial self-healing agent with a particle size of 3.35–4 mm. In studying the growth of spores in the simulated pore solution of cement-based materials, they found that the spores without immobilization treatment could not revive after 28 days of immersion in the simulated Portland cement pore solution and did not transform into vegetative cells after continued cultivation, whereas the spores protected by the carrier were still active after 28 days of immersion and could undergo the microbial morphological transformation process of "spore–vegetative cells–spore" many times, indicating that the wrapping of SC could keep the spores active in the pore solution of cement-based materials for a long time.

Another combination method is used by Zheng et al. [55], microbial spores, nutrients and SC is mixed and extruded into small particles, and then using this integrated self-healing agent in concrete, the crack healing rate of self-healing specimens reached 95% after 28 days of crack healing, and the experiments suggested that SC can serve as an excellent carrier for sprouts and can reduce the negative effect of the incorporation of microorganisms and carbohydrate-based nutrients on concrete. Xu et al. [56] selected SC mixed with 20% mass fraction silica fume as a carrier for microorganisms and found that the spores could remain active for a long time, which parallels Zheng's experimental results.

Low-alkali cementitious materials improve microbial activity by regulating the microbial microenvironment pH, thereby facilitating the mineralization process [57,58]. The remarkable results of crack self-healing in the above study also demonstrate the effectiveness of low-alkali cementitious materials for immobilizing microorganisms. However, this carrier does not favor steel protection of the matrix and acid and alkali resistance, and it affects the setting time of cement-based materials, so further improvement is needed.

4.3. Organic Materials

Organic carrier materials carry many functional groups, have diverse properties, and can be artificially controlled regarding shape (spherical, tubular, membrane, etc.), porosity, pore size, hydrophobicity, and so on [25,29]. They can stably bind microbial cells. Common organic carrier materials include fibers, rubber, and various types of hydrogels [59–62].

4.3.1. Fiber

Numerous studies have shown that fiber addition can enhance the mechanical properties and durability of cement-based materials while helping to inhibit the appearance and development of cracks in cement-based materials [62–64]. Recently, many scholars have used fibers as bacterial carriers for crack self-healing, and experiments have confirmed that combining the two can achieve a more significant crack self-healing performance.

Momina et al. [60] used coir, flax, and jute fibers as reinforcing materials and bacterial carriers. The bacteria remained in a spore state for a long time after immobilization by natural fibers. After crack formation, the spores were transformed into vegetative cells, and much calcium carbonate precipitation was produced on the three natural fibers. Compared with the other two natural fibers, flax fiber has a better protective effect on bacteria, and the healing effect of cracks outperforms that of the remaining two carriers. Singh et al. [61] used an absorbent and alkali-resistant cellulose fiber to immobilize bacteria, and the cracks healed effectively with time. The fibers accelerated the deposition of bacterial mineralization, resulting in better self-healing performance of the self-healing cement-based materials, but research on the coupling effect of fibers and bacteria and their mechanism remains limited. Su et al. [62] suggested that bacterial extracellular polymeric substances (EPS) are critical in the coupling effect of bacteria and fibers. A comparison of the calcium carbonate deposited on polypropylene (PP) fibers induced by bacteria and EPS revealed that EPS induced more calcium carbonate deposition on the fibers. The distribution of EPS was observed using fluorescent labeling (Figure 4), and the results showed that the bioorganic film formed by EPS during the repair process covered the PP fibers and calcium carbonate crystals. EPS contains charged macromolecules, such as polysaccharides, proteins, and nucleic acids, which attract cations (Ca^{2+}) from the environment through electrostatic interactions, and the fibers thus become new nucleation sites. Calcium carbonate keeps accumulating on the fiber surface, and the fibers grow and lap each other, making the space of calcium carbonate deposition change from two-dimensional to three-dimensional. This new deposition method accelerates calcium carbonate deposition, thus improving the efficiency of calcium ion utilization.

Currently, fibers are commonly used in civil engineering. Due to the high tensile strength and elongation of fibers, it can increase the tensile strength, impact resistance, shear strength, and fatigue resistance of cement-based materials, and control the further development of matrix cracks [65]. Therefore, the combination of fiber and microbial mineralization is extremely promising.

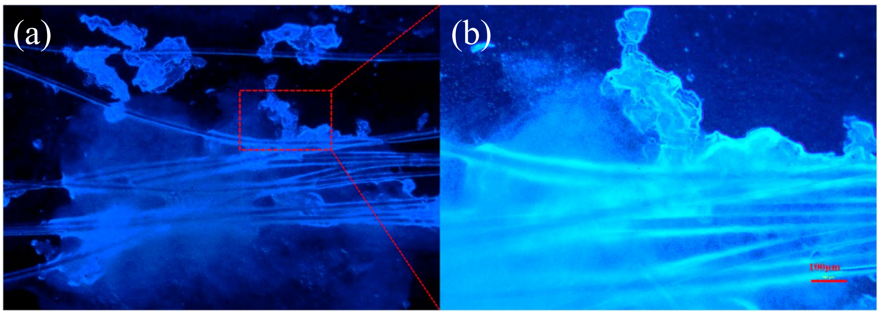

Figure 4. The optical microscope images of deposit on PP fiber [62]: (**a**) deposit induced by EPS; (**b**) biological organic film on PP fiber surface and deposits surface.

4.3.2. Rubber

Rubber particles have high porosity and a rough surface, which makes them suitable as microbial carriers. XU et al. [66] applied rubber particles made from crushed scrap tires (containing 37% natural rubber, 25% butadiene rubber, 30% carbon black, and 8% other additives) as microbial carriers and used a sodium alginate (SA) solution as a binder to adhere the microorganisms to the rubber particle surfaces. The results revealed that larger rubber particles could provide more sufficient space for bacterial growth and mineralization, their cracks healed better, and the compressive strength of rubber self-healing concrete exceeded that of ordinary rubber concrete at 28 days. However, the inherent disadvantages of rubber include relatively low stiffness and strength, and the weak bond between the rubber and the cement paste, leading to early cracking of the matrix [67], which seems to contradict the purpose of rubber as a microbial carrier.

4.3.3. PU Foam

PU foam is porous and has a low density. Bang et al. [9] first used PU foam to immobilize bacteria for repairing concrete cracks, and they observed calcite crystals embedded with bacteria by SEM analysis, suggesting that PU foam can provide protection for microorganisms from the extreme alkaline environment of concrete while acting as a nucleation site for calcite crystals. Wang et al. [10] found that PU foam immobilized bacteria maintained high ureolytic activity, and specimens containing PU foam immobilized bacteria had higher strength regain and more significant water permeability loss than reference specimens without adding PU foam and bacteria. However, PU foam is a polymeric material, which can have negative effects on the environment, and the addition of PU foam may cause a loss of concrete strength. These drawbacks are likely to be barriers to PU foam for microbial carrier materials.

4.3.4. Hydrogel

A hydrogel is a hydrophilic gel with a reticular structure that has a high-water absorption capacity and can retain large amounts of water or an aqueous solution in the reticular structure without dissolving, and the absorbed water is slowly released into the surrounding environment. The absorbing and swollen hydrogel provides a continuous supply of sufficient water for activating and maintaining bacterial activity in the cracked region, unlike the usual bacterial carriers [68,69]. Wang et al. [68] investigated the immobilization effect of a poly (ethylene oxide)-poly (propylene oxide) block copolymer (PEO–PPO–PEO) hydrogel, and the amount of urea decomposition indicated that microbial spores could remain active continuously in the hydrogel's protection. Compared with the unfixed group, the mortar specimens containing hydrogel-wrapped spores had a significant crack repair result, with a maximum crack repair width of about 0.5 mm, and the average water permeability was reduced by 68%. Hydrogels have plasticity, resist microbial degradation, and can be reused, but they can lead to a decrease in matrix strength, and their mass transfer performance needs further progress. The influence of toxic monomers in hydrogels on microbial activity requires full avoidance to maximize their performance [69–71].

4.4. Microcapsules

Microencapsulation technology is the encapsulation of one material in another, producing micron-sized capsules [72]. The encapsulation material is called the capsule wall, which is mostly organic material, and the inner material is called the capsule core, which can be solid, liquid, gas, or even active bacteria or enzymes, depending on the needs. The capsule core is protected by the capsule wall and is encapsulated for controlled release or released by some triggering mechanism. Therefore, the triggering mechanism of microcapsules is the key to realizing the self-healing of cracks in cement-based materials. The capsule wall material must have a strong sensitivity to changes in the physicochemical environment before and after the appearance of cracks and produce reactions such as rupture and dissolution to release the healing agent and achieve self-healing. Following the trigger principle, the capsule wall is divided into two types based on the physical and chemical trigger principles: the main physical trigger methods are mechanical rupture, temperature, and light triggers, and the chemical trigger methods include pH and ion triggers [73,74].

Mechanical rupture triggering dominates the triggering of microcapsules in crack self-healing in cement-based materials. Microcapsules are sensitive to changes in pH and humidity within cement-based materials, and they are flexible at high humidity or when in water and exhibit a degree of brittleness at low humidity [75]. This means that the capsule wall material can withstand the mechanical impact generated when mixing with the cement-based material to avoid damage and leakage of the healing agent caused by extrusion, and the capsule wall material can rupture in time to release the healing agent after cracks appear, triggering the repair process (Figure 5). When cracks occur in the matrix, the stress at the crack tip causes the microcapsules to rupture and release the spores, which obtain water, oxygen, and Ca^{2+} in the crack area, after which the spores sprout and produce $CaCO_3$ precipitation to repair the cracks.

Wang et al. [75] prepared microcapsules with melamine to encapsulate microbial healing agents and observed the breakage of the microcapsules when the samples were cracked under SEM. The crack healing rate and water permeability tests confirmed that the microencapsulated bacteria had a remarkable enhancement in the self-healing ability of the cracked specimens, and the crack-healing rate of the biological microcapsule group exceeded that of the non-bacterial group. Its maximum crack repair width reached 970 μm, and the water permeability resistance was about ten times that of the non-bacterial group.

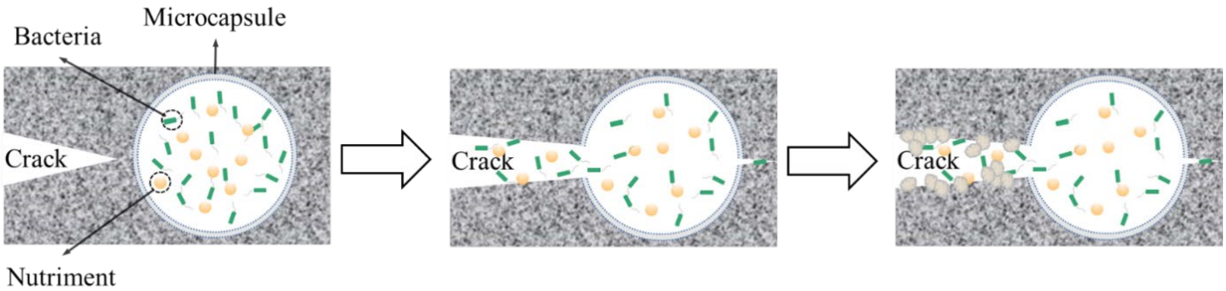

Figure 5. Schematic diagram of the self-healing process of microcapsules.

Wiboonluk et al. [76] compared three microencapsulation techniques for SA encapsulated spores: extrusion, spray, and freeze-drying. The results showed that the freeze-drying technology had the highest survival rate of bacterial budding spores (100%), whereas the squeeze and spray drying technologies had lower survival rates of 93.8% and 79.9%, respectively. Due to the operating conditions, the first two technologies easily damage the spores, whereas freeze-drying technology is used to dry the water in the sample by direct sublimation from ice in a low-temperature vacuum environment. Therefore, freeze-drying technology can maintain the original chemical composition and physical properties of the capsule wall material and stabilize the spores' potential activity, which is more suitable for microcapsule preparation.

Biomicrocapsules have excellent performance and good crack healing effects, but their high preparation cost and tedious operation process restrict the application of microcapsules in the self-healing of cracks in cement-based materials. Besides, the wall material affects the surface morphology of the microcapsules, the content of the core material, and the release effect of the core material. The synthetic organic capsule wall material has a certain biological toxicity, which harms microorganisms and the environment around the matrix, and natural organic capsule wall material has poor performance. Therefore, future microencapsulation technology should be developed toward high loading capacity, a diverse compound of capsule wall material, adjustable structure and performance, lower preparation cost, and a simpler operation process.

4.5. Other Types of Carriers

Apart from the mentioned types of carrier materials, some scholars have achieved the self-protection of bacterial communities by using bacterial flocculation to form swarms under certain conditions [77,78]. For example, Silva et al. [77] selected a highly efficient ureolytic microbial community (Cyclic EnRiched Ureolytic Powder or CERUP) as a microbial healing agent and compared it with standard *B. sphaericus* and found that CERUP had higher ureolytic activity than *B. sphaericus*. The mortar specimens exhibited good crack self-healing effects and completely healed cracks of 0.45 mm after 28 days when CERUP was mixed with the mortar without an additional protection carrier. In addition, nanomaterials have been used as microbial carriers [79,80]. Mostafa et al. [79] used iron oxide nanoparticles (IONs) with magnetic properties for bacterial immobilization, and the charged cell membranes were bound to the IONs through electrostatic interactions to complete the adsorption process. The high activity of nanoparticles promoted the hydration process of cement, significantly improved the mechanical properties of specimens, and compensated for the negative effects of nutrients on the strength of self-healing cement-based materials.

Table 2 summarizes the present state of the research and application of microbial carrier materials.

Table 2. Summary of bacterial carrier materials.

Carrier Material Type	Carrier	Immobilized Microorganisms	Immobilization Method	Immobilization Technique	Crack Healing Width (mm)	Refs
Aggregate	ED	*B. subtilis* HU58	Adsorption	Mixing extrusion molding	1.8	[6]
	EC EP	*B. cohnii*	Adsorption	Impregnated under vacuum, coating with a geopolymer	0.45 (EC) 0.79 (EP)	[7]
	Zeolite	*S. pasteurii* *S. ureae*	Adsorption	Vibrating mixing	0.1 (*S. pasteurii*) 0.07 (*S. ureae*)	[44]
	EV	*P. mucilaginosus*	Adsorption	Impregnated under vacuum, wrapping with the composite paste	0.4	[45]
	GAC	*D. nitroreducens* *P. aeruginosa*	Adsorption	Impregnated under vacuum	0.4	[8]
	Ceramsite	*S. pasteurii* ATCC 11859	Adsorption	Ceramsite pretreatment (Alkali erosion and sintering treatments), immersing	0.3	[50]
	Porous glass beads	*S. pasteurii* ATCC 11859	Covalent binding	Vibrating mixing	-	[31]
Low-alkali cementitious materials	RA	*S. pasteurii* A484	Adsorption	Impregnated under vacuum	0.28	[51]
	SC	*B. mucilaginous* L3	Adsorption	SC wraps spores and nutrients	0.5	[54]
		B. megaterium	Adsorption	Mixing extrusion molding	-	[55]
	Carbide slag etc.	*B. cereus* CS1	Adsorption	Carbide slag etc. wraps spores and nutrients	0.55	[57]
Organic materials	PVA fibers	-	Adsorption	Stir the mixture simply	-	[59]
	Coir etc.	*B. subtilis* KCTC 3135T	Adsorption	Soaked in a bacterial spore suspension	-	[60]
	Cellulose fiber	*B. subtilis*	Adsorption	Soaked in a bacterial spore suspension	-	[61]
	PP fibers	*B. alcalophilus*	Adsorption	Stir the mixture simply	0.5	[62]
	Rubber particles	*S. pasteurii* ATCC11859	Adsorption	Mixing and air-drying	0.86	[66]
		S. pasteurii ATCC 11859	Adsorption	Mixing and stirring	-	[9]
	PU foam	*B. sphaericus* LMG 22557	Encapsulation	PU prepolymer, accelerator, and bacterial suspension are mixed to form PU foam	-	[10]
	Hydrogel (PEO-PPO-PEO)	*B. sphaericus* LMG 22557	Encapsulation	Mixing of spores, nutrients, and polymer solution, adding initiator, degassing, UV irradiation, freeze grinding, and drying.	0.5	[68]
	Modified-alginate hydrogel	*B. sphaericus* LMG 22557	Encapsulation	Mixing of bacterial spore suspension with the polymer solution, adding initiator, UV irradiation, freeze grinding, and drying	-	[69]
Microcapsule	Epoxy	-	Encapsulation	Mixing and curing to form microcapsules	-	[74]
	Melamine	*B. sphaericus* LMG 22557	Encapsulation	Formation of biomicrocapsules based on polycondensation reaction	0.97	[75]
	SA	*B. sphaericus* LMG 22557	Encapsulation	Mixing and freeze-drying	0.17	[76]

Table 2. *Cont.*

Carrier Material Type	Carrier	Immobilized Microorganisms	Immobilization Method	Immobilization Technique	Crack Healing Width (mm)	Refs
Bacterial self–protection material	CERUP	-	Flocculation	-	0.45	[77]
	Nitrate reducing microbial community	-	Flocculation	-	-	[78]
Nanomaterials	IONs	*B. sphaericus* NZRM 4381 *B. licheniformis* ATCC 9789	Adsorption	-	-	[79]
	Graphite nano platelets	*B. subtilis*	Adsorption	Soaked in a bacterial spore suspension	0.81	[80]

5. Pros and Cons of Carrier Materials

As a controllable regulatory component between the interface of biotic and abiotic materials, the carrier material not only provides a new idea to solve the compatibility problem between microorganisms and cement-based materials, but also plays a key role in the development of crack self-healing technology for cementitious materials. In recent years, the use of carriers to immobilize microorganisms has been widely used in the field of crack self-healing. Existing studies have confirmed that carrier materials have significant advantages in sustaining microbial mineralization activity and improving crack self-healing. For example:

1. The immobilization process of microorganisms can be operated in batches, which makes the immobilization technology appropriate for continuous and automated industrial production. It is suitable for use in large-volume, high-consumption construction materials such as cement-based materials.
2. Avoid microbial damage due to extrusion when cement-based materials are mixed.
3. Improve the problem of lower microbial concentration caused by dilution of mixing water.
4. Enhance the resistance of microorganisms to the high pressure and high alkaline environment inside the cementitious material and keep the microorganism activity effective for a long time.
5. Increase the density of organisms, enhance the genetic stability of microorganisms and the synergy between populations [25].

The effects of each type of carrier material vary based on its unique physical structure, chemical properties and biocompatibility. In fact, the choice of carrier materials is a key factor affecting the effect of microbial mineralization and fracture repair. On this basis, the advantages and disadvantages of various carrier materials are summarized and some problem-solving methods are proposed.

5.1. Aggregates

Aggregate-type carrier materials are widely available, easy to obtain, and low cost. And most are inorganic materials such as EC, EP, EV, zeolite, etc. These materials have high physical, chemical, and biological resistance, but carry fewer functional groups and rely only on interactions between weak bonds such as van der Waals forces, hydrogen bonds, and ionic bonds to immobilize microorganisms, and have a weak binding ability with microorganisms. Although the use of adsorbents can enhance the adsorption effect of the carrier partly, the chemical composition of the adsorbent is potentially biotoxic and unfavorable to microbial activities.

In future research, aggregate-type carrier materials can gain specific groups on surface-by-surface modification or composite organic materials to obtain carrier materials with stronger binding strength and higher loading. Moreover, to address the problem of aggregate-type carrier materials that negatively affect the mechanical properties of the matrix, the high activity nanomaterials such as nano-silica, nano-TiO_2, nano-MgO, and carbon-based nanomaterials can be blended into the carrier to improve size and density [81], thereby promoting the carrier's strength and stability.

5.2. Low-Alkali Cementitious Materials

Low-alkali cementitious materials include SC, gypsum and fly ash, which have a small particle size, low alkalinity, high impermeability, and anti-frost performance. However, this kind of carrier will not only affect the setting time of cement-based materials, but also be detrimental to the durability of the matrix. Therefore, the dosage of low-alkali cementitious materials must be strictly controlled.

5.3. Organic Materials

Organic materials are a type of carrier material with many varieties. Organic materials are usually divided into natural organic materials and synthetic organic materials.

Common natural fiber carriers include coir fiber, flax fiber, jute fiber and cellulose fiber. It has characteristics of wide source, simple fixing process, non-toxic, harmless and low price. However, unlike synthetic fibers, their adsorption capacity is weak and susceptible to interference from the surrounding environment, causing a high probability of microbial shedding on the carrier's surface. Studies have shown that cellulases can modify the structure, porosity, and surface roughness of natural fibers [82]. Thus, cellulases can be used to improve the adsorption capacity of natural fibers to microorganisms and the stability of the immobilization system. To address the problem of insufficient mechanical properties of fibers (including natural and synthetic fibers), graphene materials such as graphene oxide and graphene flakes are coated on the fiber surface to improve the tensile and shear strength and interfacial properties of fibers [83].

Rubber from waste tires is made into a carrier material, which is low-cost and environmentally friendly. However, when it is mixed into concrete, it will cause the specimen to crack at an early stage. Based on the properties of fibers to inhibit the appearance and development of cracks, it is possible to consider the composite incorporation of rubber particles and fibers into concrete, and the fibers can impose constraints on the interface transition zone between rubber particles and cement paste, thereby maximizing the performance weakness of rubberized concrete.

The main feature of PU foam is its porosity and low density. Therefore, PU foamed concrete has the characteristics of good sound insulation, heat insulation, fire resistance and light weight. However, PU foam easily ages and has poor environmental compatibility. Combining PU foam with nano-silica can improve the lack of biocompatibility and stability of a single PU foam carrier and combine the good mechanical properties of nano-silica to increase the impact resistance of PU foam [84].

The hydrogel has good water absorption and moisture absorption ability, and can maintain the high activity of microorganisms, but the strength of the matrix will be lost after being incorporated into the matrix. At present, hydrogels are mainly modified by physical modification, graft copolymerization and radiation polymerization [85], such as polyacrylic superabsorbent polymer hydrogel, poly (acrylamide-*co*-sodium alginate) hydrogels, and other modified hydrogels [86,87]. It has high mechanical properties and can improve the compressive strength and durability of concrete.

5.4. Microcapsules

Microcapsules are able to isolate microorganisms from the internal environment of cementitious materials. It provides better protection against microorganisms than other carrier materials. However, the application of microcapsules for microbial immobilization in cement-based materials is not common due to their high cost and complicated preparation methods. A future development direction can be considered in terms of reducing their cost.

5.5. Other Types of Carriers

Nanomaterials exhibit new properties including microscopic size effects, surface effects, filling effects, and adsorption properties that macroscopic materials lack [81]. The use of nanomaterials can regulate the hydration process of cement, which in turn improves the mechanical properties and durability of the hardened paste. However, nanomaterials are easily agglomerated and not easily dispersed, and in addition, they are limited by cost. In addition, studies on the use of microbial communities as protective carriers for crack self-healing are not common at present, mainly due to immature technology.

6. Conclusions and Prospect

This paper summarizes the research progress of microbial carrier materials, classifies them according to their morphology and properties, clarifies the corresponding mechanisms of action, and concludes with the advantages, disadvantages, and healing effects of each. However, the application of carrier materials in practical construction engineering is limited by the complexity of the internal environment of cement-based materials, the stability of carrier materials, the lifetime of microorganisms, immature microbial immobilization conditions, and process design for large-scale industrial production, etc. The research and development of new technologies and multidisciplinary cross-fertilization are imperative for developing carrier materials, which can be improved in the following aspects:

1. Cement-based materials in service: the carrier material must maintain good compatibility with microorganisms to ensure the long-term effective activity of microorganisms and be able to respond quickly to various small cracks and defects while avoiding interference from other external factors to control the release of microbial self-healing agents. Simultaneously, the durability of the carrier material in use and its long-term effect on the matrix and external environment also require consideration. Therefore, how to improve the preparation process of carrier materials and enhance their sustainability and stability should continue to receive attention and development.
2. In the above studies, solid waste resources (e.g., RA), waste tires, and plant fibers (e.g., coconut coir and flax fibers) have demonstrated excellent immobilization effects. In the future, the rational use of solid waste resources and renewable natural materials and the strengthening of research on environmentally friendly carrier materials will remain significant development trends.
3. Presently, most studies on carrier materials mainly focus on a single type of material. Single carrier materials are highly targeted but have poor comprehensive performance. By synergizing composite carrier materials, such as inorganic–organic combinations, inorganic materials with porous structures and organic materials with more functional groups (e.g., amide, ether, etc.) combined, makes carrier materials with higher loading and stronger immobilization effects and may be beneficial to the growth and reproduction of microorganisms. In the future, single carrier materials are modified so that each type of carrier material can complement the other to obtain newer functions and finally new composite carrier materials with better performance to address the problems of poor mass transfer performance, low mechanical strength, and unstable bonding strength of single carrier materials.
4. With the development of tissue engineering and molecular biology, carrier materials can be endowed with targeting functions through surface modification in the future. When the cement-based material is cracked, the carrier with targeting ability is stimulated by external moisture and oxygen and enriched at the crack through connecting pores, which makes the microorganism form a relatively high concentration in the crack area, thus effectively improving the crack repair effect.
5. The parameter values, such as loadings, distribution area, working conditions, and morphological changes of the carrier, which are not easily observable, indirectly affect the crack healing effect. Linking such problems with computer simulation

techniques through numerical simulations, combined with finite element or finite volume concepts, is imperative to assess and predict crack self-healing effects.

Author Contributions: Writing—Original, C.F. and X.Z.; writing—Review and editing, C.F., B.C., X.Z. and H.G., translation, W.Z. and J.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Public Welfare Special Project of Henan Province (201300311000), Henan Provincial Department of Science and Technology Research Project of China (212102310566, 212102310564) and Henan Outstanding Foreign Scientists' Workroom (GZS2021003).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be shared upon direct request.

Conflicts of Interest: The authors declare no conflict of interest. matrix durability, and crack development.

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