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Consolidation of archaeological gypsum plaster by bacterial biomineralization of calcium carbonate ☆

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Gypsum plasterworks and decorative surfaces are easily degraded, especially when exposed to humidity, and thus they require protection and/or consolidation. However, the conservation of historical gypsum-based structural and decorative materials by conventional organic and inorganic consolidants shows limited efficacy. Here, a new method based on the bioconsolidation capacity of carbonatogenic bacteria inhabiting the material was assayed on historical gypsum plasters and compared with conventional consolidation treatments (ethyl silicate; methylacrylate–ethylmethacrylate copolymer and polyvinyl butyral). Conventional products do not reach in-depth consolidation, typically forming a thin impervious surface layer which blocks pores. In contrast, the bacterial treatment produces vaterite ($CaCO_3$) biocement, which does not block pores and produces a good level of consolidation, both at the surface and in-depth, as shown by drilling resistance measurement system analyses. Transmission electron microscopy analyses show that bacterial vaterite cement formed via oriented aggregation of $CaCO_3$ nanoparticles (~20 nm in size), resulting in mesocrystals which incorporate bacterial biopolymers. Such a biocomposite has superior mechanical properties, thus explaining the fact that drilling resistance of bioconsolidated gypsum plasters is within the range of inorganic calcite materials of equivalent porosity, despite the fact that the bacterial vaterite cement accounts for only a 0.02 solid volume fraction. Bacterial bioconsolidation is proposed for the effective consolidation of this type of material. The potential applications of bacterial calcium carbonate consolidation of gypsum biomaterials used as bone graft substitutes are discussed.

1. Introduction

Gypsum-based mortars and plasters have been used since the advent of pyrotechnology (from around 12 000 BC) [1] in architectural applications as joint mortars, to cover masonry or to support paintings and decorations [2]. Gypsum was a rather common building and decorative material in the Levant [1] and ancient Egypt [3,4], as well as in the Mediterranean countries, especially during the Middle Ages [2,5]. There are numerous examples of medieval Islamic architecture where gypsum was cast and/or carved as highly sophisticated decorative structures, such as the *mocarabes* from the Alhambra in Granada, Spain (twelfth to fifteenth centuries) (Fig. 1A). Up to now, gypsum has been widely used in plasterworks found in old and modern buildings worldwide [6–8].

In addition to its applications as a building material, calcium sulfate has been used as a ceramic bone graft substitute for more than 100 years [9–11]. This biomaterial has been applied for bone repair either as a self-setting paste or as preset solid bodies (blocks or pellets) [10,12]. Its biomedical applications include treatment of bone defects, endodontic surgery, guided tissue regeneration, sinus augmentation and drug delivery [10]. Among the main advantages of calcium sulfate as a biomaterial are its high biocompatibility, rapid and complete resorption and remarkable osteoconductivity [10]. However, its low strength and rapid resorption can be disadvantageous in some circumstances [10–12]. This has prompted its use as a composite biomaterial in combination with inorganic (e.g., calcium phosphates, carbonates and/or silicates) and organic materials (e.g., carboxymethylcellulose, gelatin, hyaluronic acid, chitosan and polyacrylic acid) [10–13].

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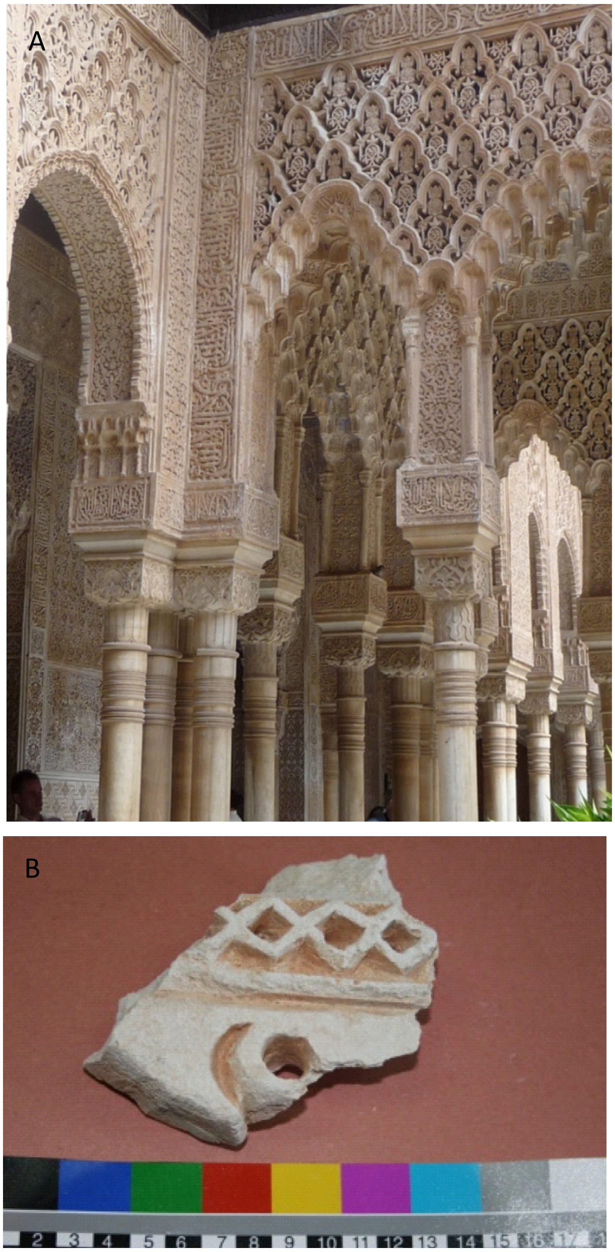

Fig. 1. Ancient gypsum plasterwork: (A) example of decorative gypsum plaster at the Lionś Court in the Alhambra (Granada, Spain); (B) representative decorated gypsum plaster recovered from the archaeological site "Alcázar de Guadalajara" (Guadalajara, Spain).

Gypsum-binder technology involves an initial thermal treatment of gypsum (heating to ~120–160 °C) to form the hemihydrate (the mineral bassanite, also called "plaster of Paris") according to the reaction [7]:

$$CaSO_4 \cdot 2H_2O \leftrightarrow CaSO_4 \cdot 0.5H_2O + 1.5H_2O \quad (1)$$

When the hemihydrate is mixed with water, the fresh mix sets and rapidly hardens via the formation of an interconnected porous structure of acicular and/or prismatic gypsum crystals that impart strength to the plasterwork (i.e., Reaction (1) is reversible) [14]. Gypsum is thus suitable for many architectural and decorative applications because, in addition to its fast setting, it possesses high plasticity, good adhesion, ease of application and adequate protective behavior. However, its softness (Mohs hardness of 2), poor mechanical behavior (typical values for gypsum plaster with ~30–50% porosity are: ~4–7.5 GPa Young's modulus, ~7–18 MPa uniaxial compressive strength, ⩽3 MPa uniaxial tensile strength) [14,15] and relatively high solubility (pK_{sp} = 4.62) [16], favor its degradation, especially when exposed to humidity (rain, condensation and/or rising damp) [5]. The implementation of conservation strategies, typically including consolidation, is thus needed to limit deterioration [8,17]. However, consolidation of artistic and historical gypsum plaster is a poorly explored and challenging issue. Consolidation could be achieved through the application of organic or inorganic consolidants commonly used in stone conservation [18,19]. This is the case of acrylic, epoxy or (poly)vinyl polymers, and their copolymers [4]. However, polymer protectives and consolidants are generally incompatible (physically and chemically) with the inorganic substrate (i.e., stone or plaster) they are applied to, and may result in exacerbated damage [20]. Alkoxysilanes have also been applied for consolidation, especially on silicate substrates such as sandstones [21]. The formation of amorphous silica upon sol–gel transition offers some advantages as the end product is inorganic and can act as a new cement. However, the formation of superficial films and/or hard crusts [20], drying-induced cracking of the silica gel [22,23] and poor bonding with non-silicate substrates [16] may undermine the effectiveness of such a treatment. Recently, nanolimes have been proposed as a consolidant for gypsum stucco [24], yet little is known about its effectiveness. To these shortcomings, one has to add economic, environmental, health and safety issues that must be considered in determining the suitability and compatibility of a given treatment [25].

An alternative to standard chemical treatments is the use of the carbonatogenic capacity of some bacteria to consolidate/protect buildings and decorative inorganic materials [26–33]. It is an environmentally friendly bioconsolidation strategy based on the capacity of bacteria to induce the new formation of calcium carbonate biocement within the porous system of materials [28]. Bacterially induced mineralization occurs because bacteria can change the chemistry of their environment as a result of their metabolic activity, and their cell structures and debris, as well as the exopolymeric substances (EPS) they secrete, can act as nuclei for heterogeneous $CaCO_3$ crystallization [28,29,34]. In the last few decades, bacterial biomineralization processes and their application for the conservation of ornamental stones have been studied by a number of researchers (see reviews in Refs. [35,36]). Gonzalez-Muñoz et al. [37] developed a new bioconsolidation method based on the selective activation of carbonatogenic microbiota inhabiting stone by the application of a suitable nutritional solution. Both the airborne bacteria as well as the stone-inhabiting carbonatogenic microorganisms can be stimulated to grow and to induce the in situ formation of new calcium carbonate biocement, which effectively consolidates decayed stones [29,30]. This last method possesses the principal advantage that it does not require the application of a bacterial culture, thereby circumventing any potential problem/complexity associated with the culture and application of foreign bacteria for calcium carbonate production.

Despite the numerous studies on bacterial carbonatogenesis, little is known about gypsum plaster consolidation using bacterial calcium carbonate precipitation. To our knowledge, only one paper reports on the application of a commercial bacterial conservation treatment (*Bacillus cereus* cultures) on laboratory-made gypsum plaster samples [38]. However, the aim of such a study was to unambiguously identify bacterial $CaCO_3$ precipitates. No attempt to evaluate the effectiveness of the treatment in terms of consolidation efficacy was made. Up to now, the application of this new consolidation strategy was focused on the conservation of carbonate stones [29,30] and sandstones [39], as well as on the protection and repair of concrete and cement structures [40]. Little is known on the influence of a gypsum substrate on bacterial $CaCO_3$ precipitation, on the effectiveness of the bacterial conservation treatment

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