



Recent Advances in Silk Sericin/Calcium Phosphate Biomaterials

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OPEN ACCESS

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Specialty section:

This article was submitted to
Biomaterials,
a section of the journal
Frontiers in Materials

Received: 19 September 2019

Accepted: 21 January 2020

Published: 18 February 2020

Citation:

Veiga A, Castro F, Rocha F and
Oliveira AL (2020) Recent Advances in
Silk Sericin/Calcium Phosphate
Biomaterials. *Front. Mater.* 7:24.
doi: 10.3389/fmats.2020.00024

Calcium phosphates (CaPs) have been widely used in the field of biomedical engineering as bone graft substitutes or as carriers for drug delivery applications. Recent developments have focused on combining CaPs with proteins to obtain functional biomaterials that accommodate a broader spectrum of functional requirements. Silk sericin was considered an unutilized protein by-product from the textile industry, generating tons of residues every year. However, much effort has been dedicated to its recovery after being associated with numerous biological properties such as antioxidant, antibacterial, anti-coagulation and regenerative activities. In the past years, sericin has also demonstrated to be suitable as a template for CaP mineralization. The present review focuses on the recent developments for the production of sericin/CaP composites, exploring their potential applications in bioengineering and opening new avenues in other research fields such as in the cosmetic, food and environmental sectors. In addition, this paper can also be useful as a guideline to design future research based on sericin/CaP biomaterials.

Keywords: biomaterials, bone tissue engineering (bone-TE), calcium phosphates (CaPs), drug delivery, hydroxyapatite (HAp), sericin

INTRODUCTION

Bone matrix consists of both a non-mineralized organic component (~20% of the wet weight) and a mineralized inorganic component (~65–70%), composed mainly by collagen and carbonated calcium phosphate (CaP) crystals, respectively. Crystallographic *c*-axis of these crystals are arranged parallelly to the longitudinal axis of the fibrous protein, forming a tough and flexible nanocomposite structure (Fuchs et al., 1997; Amini et al., 2012).

Accordingly, calcium phosphates (CaPs) from natural or synthetic nature, are widely used in bone-related applications due to their biocompatibility, low density, chemical stability and crystallinity (Vallet-Regí and González-Calbet, 2004). Among existing CaPs, hydroxyapatite (HAp) is the most studied biomaterial due to its close similarity to human hard tissue in terms of morphology and composition (Habraken et al., 2016).

In a quest to mimic bone tissue, several studies have focused on the synthesis of CaP composites using synthetic polymers [polyglycolic acid (PGA), poly L-lactic acid (PLLA), polylactic-coglycolic acid (PLGA), and polycaprolactone (PCL)] (Rezwan et al., 2006; Lee and Yuk, 2007; Chen et al., 2014), natural polymers (alginate, chitosan, cellulose) (Pighinelli and Kucharska, 2013; Cardoso et al., 2014; Salama, 2019), proteins (collagen, fibrin, keratin, and silk fibroin) (Dias et al., 2010; Bleek and Taubert, 2013; Brown and Barker, 2014; Liu, 2015; Farokhi et al., 2018),

or combinations of the foregoing (Chen et al., 2014). Synthetic polymers may induce local and systemic host reactions due to the release of chemicals and monomers from polymer degradation, while natural polymers may lead to products with different characteristics due to the differences in raw materials (Chen et al., 2014).

Proteins have demonstrated to regulate the size, particle size distribution, morphology, and assembly method of nanostructured CaPs, being thus used to fabricate novel materials with biomimetic characteristics for hard tissue repair (Cai and Yao, 2010; Chen et al., 2014). Further, their natural origin confers unique biocompatibility, versatility, and biodegradability properties (Swetha et al., 2010). Collagen has been conventionally used in the synthesis of organic/CaP composites. Although collagen/CaP composites have a greater similarity to natural bone, the clinical application of collagen-type biomaterials is still limited due to its high cost, increased risk of cross-infection and fast degradability (Chen et al., 2014). Alternative protein systems can play a pivotal role during the mineralization of CaP, as a binding-matrix. Keratin/CaP and fibrin/CaP composites have biocompatibility and bioactivity properties suitable for bone tissue engineering. However, these biomaterials lack mechanical properties and processing properties, and have fast biodegradability (Gsib et al., 2017; Shavandi et al., 2017).

In this context silk is a valuable alternative due to its excellent intrinsic properties such as non-toxicity, biodegradability, self-assembly, mechanical stability and controllable structure (Altman et al., 2003; Ha et al., 2013). In nature, silk-like proteins are produced by several organisms such as silkworms, spiders, mollusks, scorpions, bees, and ants (Holland et al., 2019). The use of silk from the domesticated silkworm *Bombyx mori* is well-established as suitable for biomedical applications due to its abundance, batch-to-batch stability, and clinical track record (Ude et al., 2014). This type of silk comprises a fibrous semi-crystalline silk core, silk fibroin, which is mainly responsible for the load-bearing capacity, and an outer layer of a globular protein, sericin, which serves as gluing agent and has a protective function. While fibroin is processed in large scale in the textile industry, sericin is a by-product generated during this process, in the so-called degumming procedure (Mondal et al., 2006).

Silk fibroin has been widely used and investigated for applications such as sutures (Saxena et al., 2014), artificial ligaments (Farè et al., 2013), tissue engineering constructs (Kasoju and Bora, 2012), and substrates for cell culture (Liu et al., 2012). Fibroin/CaP composites have been reported and are considered suitable for load bearing applications (Yan et al., 2013; Farokhi et al., 2018).

Silk sericin, on the other hand, was until recently considered unfit for biomedical use. Of 1 million tons of silkworm cocoons produced each year worldwide, ~50,000 t of sericin are generated, leading to environmental and economic concerns (Aramwit et al., 2012b). This protein, in its native state, was found to elicit immune and allergic responses when present in virgin silk sutures (Soong and Kenyon, 1984). However, emerging evidence suggests that extracted sericin *per se* is biocompatible, similarly to silk fibroin or collagen (Chirila et al., 2013; Lamboni

et al., 2015). Therefore it has been gaining reputation in the biomedical field with several works showing its potential, as revised elsewhere (Kunz et al., 2016; Ahsan et al., 2018). Thus, the use of sericin/CaP composites not only reduces the inherent environmental impact of sericin disposal but also allows the development of new functional biomaterials. This silk protein increases antioxidant, anti-tyrosinase and anti-inflammatory activity; stimulates collagen production, tumor inhibitory effects; induces the nucleation of bone-like CaPs; and promotes stability and prolonged release in drug delivery systems (Aramwit, 2014; Lamboni et al., 2015).

The molecular weight distribution and even the amino acid composition of sericin were found to be dependent on the extraction method used (Yang et al., 2014c; Kunz et al., 2016). Sericin consists of 18 amino acids, among which serine, histidine, glutamic acid, aspartic acid, threonine, and tyrosine are normally present in higher percentages. Thus, this protein is highly hydrophilic and its molecular weight can range from 20 to 400 kDa (Kunz et al., 2016). Its polar side chains have several functional groups (carboxyl, amine, hydroxyl) responsible for moisturizing and oxidizing properties, allowing the interaction with other compounds through crosslinking, copolymerization or blending to form improved biomaterials (Ahsan et al., 2018). According to the literature, amino acids can highly influence the mineralization process of CaPs. Glutamic acid and aspartic acid play a critical role in controlling HAp nucleation and growth, by attracting calcium and phosphate ions and consequently increasing the local supersaturation, which results in the development of crystals. Histidine and other negatively charged amino acids are involved in HAp nucleation within extracellular matrix proteins (George and Veis, 2008; Tavafoghi and Cerruti, 2016).

Sericin mainly adopts the form of an amorphous random spiral and may also present the form of a β -sheet organized structure. The random spiral easily acquires β -sheet conformation as a consequence of moisture absorption and mechanical elongation, forming a denser, organized, crystalline and less soluble structure. If the protein is dissolved in hot water and subsequently undergoes a temperature decrease, its random coil structure becomes β -sheet, acquiring a gelatinous form (Chen et al., 2015). That is, with the change in temperature the conformation of the protein changes. Upon heating the sericin dissolves in solution, upon cooling the sericin solution jellifies (Sol-Gel properties; Kunz et al., 2016).

SILK SERICIN/CALCIUM PHOSPHATE-BASED MATERIALS

Recently, sericin has demonstrated to be suitable as a template for CaP mineralization. In the literature, sericin/CaP biomaterials are usually synthesized by wet mechanochemical processes, where mineralization occurs by spontaneous nucleation (Cai et al., 2009, 2010) or using a surface as template (Takeuchi et al., 2005b; Yang et al., 2014a). The resulting composites generally present a poor crystallinity and the size of the particles generated can range from 20 to 500 nm in length to 3–80 nm

in width (Cai et al., 2009, 2010; Veiga et al., 2019). The influence of several experimental conditions on the formation of these sericin/CaPs has been studied by several authors, namely different sericin extraction and storage procedures, mineralization time, sericin concentration, and temperature (**Table 1**). In the works of Takeuchi et al. (2003a, 2005b), sericin was obtained by extraction in boiling water, using different temperatures and storage procedures. It was found that high molecular weight sericin and β -sheet structure induces apatite deposition. These conditions were achieved for higher extraction temperatures and sericin storage. It is well-known that the extraction method used influences the molecular weight and the amino acid concentration of sericin, which results in different physical and biological properties (Kunz et al., 2016). According to Aramwit et al. (2010), extraction in boiling water is the least toxic procedure to cells and activates the highest collagen production. Acid, alkaline, and urea extraction methods can also be used to obtain sericin that promotes the formation of bone organic matrix. However, these approaches can result in toxicity at higher sericin concentrations (Padamwar and Pawar, 2004). Moreover, acid and alkaline methods have a degrading effect on proteins and considerably increase its solubility. Although urea and enzymatic methods can be used to obtain unchanged sericin, the procedures are expensive and time consuming. On the other hand, hot-water extraction is a simple and environmentally friendly method that preserves the main characteristics of silk sericin (Gulrajani et al., 2000; Dash et al., 2008).

As for the influence of the mineralization time on apatite deposition, it was found that the optimum days for growth of nano HAp crystals with low crystallinity is 7 days. In contrast, after 30 days, the size of HAp crystals increases, and other CaP phases are formed (Sukjai et al., 2012). Further, it was also shown that nucleation of HAp mediated by sericin is a gradual process, since nanoneedle crystals with low crystallinity are only grown after at least 24 h of mineralization (Yang et al., 2014b). In the work of Zhang et al. (2014), the same conclusions were drawn for ethanol-treated sericin films immersed in SBF. A three-dimensional (3D) structure with globular carbonate apatite particles was observed after 7 days. Further, rigid and brittle properties of the film increased with mineralization time, since apatite particles were increasingly being deposited. While the elastic modulus increased significantly after 5 and 7 days (23.027 ± 0.83 to 57.02 ± 1.04 MPa), the tensile strength (day 1: 1.58 ± 0.53 , day 7: 0.24 ± 0.18) and the elongation at break of the 3D film decreased (day 1: $112.83 \pm 49.02\%$, day 7: $2.01 \pm 1.81\%$). The increase of the mineralization time and sericin concentration was also reported to lead to an increase in the mean particle size of the particles synthesized (Cai et al., 2009). The recent developments in sericin/CaP composites and its potential for biomedical applications is presented in the next section.

RESEARCH APPLICATIONS

Biom mineralization

Although proteins play a crucial role during mineral formation in biological systems, the biom mineralization process is still far from being understood (Subburaman et al., 2006). Therefore,

most studies on sericin/CaP composites focus on contributing to the understanding of this phenomenon. Sericin/CaP nanoparticle systems obtained are generally nanorod-like crystals with poor crystallinity. Further, sericin promotes crystal growth of HAp along the c-axis, resembling natural biomineralization. Although in the work of Cai et al. (2010), HAp particles similar to mineral bone were achieved in the absence of sericin, this was only verified for specific temperature and pH conditions. Other works argue that the presence of sericin promotes a homogeneous assembly of HAp, under several experimental conditions (Cai et al., 2009; Yang et al., 2014b). The proposed mechanisms for the assembly of CaP crystals in the presence of sericin are usually based on changes in the protein conformation. When dissolved, sericin adopts an amorphous structure in which the hydrophilic sidechains are exposed. However, when submitted to different triggers (e.g., mechanical stretching properties, moisture absorption, temperature, dehydration, or using crosslinking agents), changes in random coil structure for β -sheet easily occur (Teramoto and Miyazawa, 2003; Nayak et al., 2012; Kunz et al., 2016).

According to Takeuchi, the induction sites for HAp nucleation in sericin films are governed by the arrangement of carboxyl groups on the protein (Takeuchi et al., 2005b). When a protein acquires a β -sheet conformation, the amino acid side chains alternate between the two faces of the sheet. Hydrophobic side-chains point in one direction and polar side-chains in the other, which can explain orientation of functional groups in β -sheet sericin (Boyle, 2018). For sericin/HAp particles, the presence of chelated calcium ions in the precipitation medium provides nucleation sites. The formed HAp crystals are rearranged along their c-axis by attaching to the sericin molecular chains (Cai et al., 2009).

The understanding of the biomineralization process can lead to numerous applications in biomedical engineering, namely for the development of advanced bone substitutes (Chen et al., 2019; Veiga et al., 2019). Even though pure sericin has poor mechanical properties, which hinders its utilization in bone tissue engineering (bone-TE), its biological properties can promote bone formation and induce the nucleation of bone-like HAp. In fact, sericin/CaP materials have shown the ability to promote cell differentiation and proliferation of bone marrow-derived mesenchymal stem cells (BMSCs) and of human osteosarcoma cells (MG-63; **Table 1**). Therefore, most of the developed works on sericin/CaP focus on demonstrating the potential of these composites in the field of bone-TE, as biomaterials for bone filling and repair or as drug delivery systems for tumor therapy, as illustrated in **Figure 1**.

Bone Tissue Engineering

Although some authors mention the potential of sericin/CaP as scaffolds for TE (Takeuchi et al., 2003b; Yang et al., 2014b), an ideal 3D scaffold should not only act as a template for tissue growth and has controlled resorbability, but should also elicit a regenerative effect and present adequate mechanical performance over time (Canillas et al., 2017). As previously mentioned, the last requirement is not fulfilled by using only sericin (Ahsan et al., 2018).

TABLE 1 | Articles on sericin/CaP biomaterials.

Objectives	Methodology	Experimental conditions	Studied variables	Physicochemical properties	Biological properties	Applications	References
Investigate the ability of natural silk and its related materials to facilitate apatite deposition under biomimetic conditions.	<ul style="list-style-type: none"> - Sericin extraction was performed in boiling water (105°C, 1 h); - Soaking method: - Cloths made of raw silk, normal silk fiber, and sericin films were soaked in 1.5 SBF. 	pH = 7.25; T = 36.5°C; Mineralization time: 7 days.	-	<ul style="list-style-type: none"> - The deposition of apatite microspheres was observed on both the surface of cloth made from raw silk fiber and in the sericin films; - CaP particles were not obtained on cloth made from normal silk fibers; - The apatite deposition on the raw silk fiber cloth was accelerated by presence of calcium ions (treatment with CaCl₂). 	-	Present the potential of sericin/HAp composites in the field of bone tissue engineering (as bone substitutes and scaffolds).	Takeuchi et al., 2003b
Study the structural effect of sericin on its apatite-forming ability using 1.5 SBF.	<ul style="list-style-type: none"> - Soaking method: - Sericin films were immersed in 1.5 SBF. 		<p>Four Different types of sericin films were obtained:</p> <p>Extraction/Storage of sericin</p> <p>105°C 1 h/none: 105–0 d; 120°C 1 h/none: 120–0 d; 105°C 1 h/4°C for 2 weeks: 105–2 w; 120°C 1 h/4°C for 2 weeks: 120–2 w.</p>	<ul style="list-style-type: none"> - Molecular weight: 134, 106, 42, and 43 kDa for 105–0 d, 105–2 w, 120–0 d, and 120–2 w, respectively; - After storage for 2 weeks at 4°C, β-sheet structure became dominant; - Spherical HAp particles with low crystallinity were only observed on the surface of 105–2 w film after 7 days. 	-	Contributing for the understanding of biological mineralization.	Takeuchi et al., 2003a, 2005b
Investigate the optimum day for the growth of HAP in sericin-coated silk fibers using SBF.	<ul style="list-style-type: none"> - Soaking method: - Sericin coated silk fibers and non-sericin coated silk fibers were used were soaked in SBF of various concentrations. 	T = 37°C.	<p>Mineralization time: 7 and 30 days.</p> <p>SBF concentration (1.5 and 1.0 SBF).</p>	<ul style="list-style-type: none"> - In non-sericin coated silk fibers, flat shape CaP crystals were formed after 7 days. With the increase in SBF concentration, cauliflower shape HAp crystals were obtained; - In sericin coated silk fibers only cauliflower shape HAp crystals were formed for 1.0 SBF concentrations (1–4 μm) and 1.5 SBF (0.1–0.2 μm). 	-	Contributing for the understanding of biological mineralization.	Sukjai et al., 2012
Investigate the mineralization of sericin by alternative soaking in calcium and phosphate.	<ul style="list-style-type: none"> - Sericin (wt.%) = 8, extraction was performed in boiling water for 30 min; - The sericin film was soaked in 90% (v/v) ethanol; - Alternative soaking of the sericin film in calcium and phosphate solutions: 1° [CaCl₂] (100 mM); 2° [NaHCO₃] (60mM). 	pH = 8.7; T = 37 °C.	Immersion cycles: 5, 10, 15 cycles.	<ul style="list-style-type: none"> - When the cycles of dip coating were set to be 5, 10, and 30, respectively, aggregation appeared on the coating and apatite spherical crystals were increased; - The coating thickness increased by increasing cycles of dip coating. 	- <i>In vitro</i> assays showed that the HAp/sericin composites obtained have higher cell adhesion and growth activity of MG-63 cells, when compared to HAp.	Present the potential of HAp/sericin composites in the field of bone tissue engineering (for bone regeneration).	Yang et al., 2014a

(Continued)

TABLE 1 | Continued

Objectives	Methodology	Experimental conditions	Studied variables	Physicochemical properties	Biological properties	Applications	References
Study the mineralizing of ethanol-treated sericin film in SBF.	Sericin (wt.%) = 9, extraction was performed in boiling water for 30 min; - The sericin film was soaked in 40% (v/v) ethanol; Soaking method: - The sericin/ethanol film was immersed in 1.5 SBF.	pH 7.4; T = 37 °C.	Mineralization time = 1, 3, 5, or 7 days.	- With the increase in mineralization time, the quantity of the deposited inorganic substance increased, showing a 3D aggregated structure; - After 7 days globular carbonate apatite particles with low crystallinity were observed (initial thickness = $93.5 \pm 728.7 \mu\text{m}$; thickness after 7 days: $6.22 \pm 2.13 \text{ mm}$).	<i>In vitro</i> assays showed that the sericin/HAp composites obtained have higher cell viability of human osteosarcoma MG-63 cells, when compared to sericin.	Present the potential of sericin/HAp composites in the field of bone tissue engineering (as 3D materials).	Zhang et al., 2014
Study the effect sericin concentration and mineralization time on HAp assembly.	Co-precipitation method: $[\text{Na}_2\text{HPO}_4]$ (12 mM); $[\text{CaCl}_2]$ (20 mM) + sericin.	pH=9.5; T= 25 °C.	Mineralization time: 0, 20, and 60 min; Sericin (w/v%) = 0.001, 0.1, 0.5, and 1, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China).	- Rod-like sericin/HAp composites were obtained; - The crystallinity of the composites is lower when compared to HAp; - Higher concentration of sericin led to the synthesis of larger HAp particles—sericin 0.001% had the smallest size of 50–100 nm in length and <20 nm in diameter, while sericin 1% possessed the largest size of 300–500 nm in length and 50–80 nm in diameter; - Longer mineralization time led to an increase of the crystallinity and size of the composites; - Size of the crystals goes from 50 to 100 nm in length and <20 nm in diameter; to 300–500 nm in length and 50–80 nm in diameter.	<i>In vitro</i> assays showed that the composites obtained have higher cell differentiation and proliferation of MG-63 cells, when compared to HAp. Further, composites with higher sericin concentration had the highest ability to accelerate cell proliferation and differentiation.	Contributing for the understanding of biological mineralization.	Cai et al., 2009
Study the effect of the solution pH value on HAp assembly.	Sericin (w/v%) = 0.625, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China). Co-precipitation method: $[\text{Na}_2\text{HPO}_4]$ (30mM); $[\text{CaCl}_2]$ (12.5 mM) + sericin.	T=50 °C; Mineralization time: 320 min.	pH: 7.4, 10, and 12.	- A homogeneous assembly of HAp with an enamel prism-like structure could be obtained both in the presence of sericin and in its absence (T =50°C and pH 10); - At pH 7.4, few aggregated nanoplates were formed; - When the pH value increased to 10, a large amount of nanoparticle bundles was obtained. - At pH 12 the particles have a size of 150–250 nm in length and 50–80 nm in width. The particles obtained have rod-like morphology; - All the particles have low crystallinity, being the samples with sericin more amorphous.	-	Contributing for the understanding of biological mineralization.	Cai et al., 2010

(Continued)

TABLE 1 | Continued

Objectives	Methodology	Experimental conditions	Studied variables	Physicochemical properties	Biological properties	Applications	References
Study the effect of sericin concentration and mineralization time on HAp assembly and its osteogenic potential.	Sericin (mg/mL) = 0.5, 2, and 8; extraction was performed in boiling water (120 °C for 30 min). Co-precipitation method: [Na ₂ HPO ₄] (12 mM); CaCl ₂ (20 mM) + sericin.	pH = 9.5; T = room temperature.	Mineralization time: 2, 6, 12, and 24 h.	<ul style="list-style-type: none"> - A higher concentration of sericin promotes the formation of nanoneedle-like crystals; - Nucleation of HAp mediated by sericin is a gradual process; - After 2 h, a clump of starfish-like crystals was randomly scattered; - After 6 h, spherical crystals were formed and aggregated; - After 12 h, spindly crystals were heavily aggregated; - After 24 h nanoneedle crystals with 20–40 nm in length and 3–5 nm in width were obtained. 	<i>In vitro</i> assays showed that the mineralized sericin/HAp composites obtained have higher osteogenic differentiation of BMSCs, when compared to non-mineralized composites.	Present the potential of sericin/HAp composites in the field of bone tissue engineering (as scaffolds).	Yang et al., 2014b
Present a new simple method to synthesize sericin/HAp composites and study the effect of SS concentration on HAp assembly.	Co-precipitation method: [Na ₂ HPO ₄] (0.012 M); [CaCl ₂ ·2H ₂ O] (0.02 M) + sericin.	T = 37°C; pH ≈ 6; Mineralization time: ≈ 2 h 30 min.	Sericin (g/L) = 0.1, 1, extraction was performed in boiling water (1 h, 150 rpm).	<ul style="list-style-type: none"> - The synthesized particles are single-phased nanometric HAp with low crystallinity; - The increase in sericin concentration is associated with decreased crystallinity; - Sericin/HAp composites have a higher mean particle size when compared to HAp. Further, the increase in sericin concentration leads to a size increase (HAp—0.070 μm; sericin 0.1 g/L—0.105 μm; sericin 1 g/L—0.116 μm.); - The increase in sericin concentration also leads to the formation of more particles with a plate-like structure. 	<i>In vitro</i> assays showed that the sericin/HAp composites obtained do not elicit a cytotoxic response on human primary cells (HNDFs) and that at high concentrations (100 mg/mL) elicit a lower cytotoxic response than a commercially available HAp.	Contributing for the understanding of biological mineralization.	Veiga et al., 2019
Use sericin to regulate the mineralization of CaCO ₃ .	Sericin (g/L) = 2, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China). Co-precipitation method: [Na ₂ CO ₃] (0.2 mol/L); CaCl ₂ (0.2 mol/L) + sericin.	T = 20°C.	Mineralization time = 10, 20, 30, 40, and 50 min.	<ul style="list-style-type: none"> - With the increase in the mineralization time, the crystal phase of CaCO₃ transferred from calcite dominated to vaterite dominated mixtures; - The morphology of CaCO₃ changed from disk-like calcite crystal to spherical vaterite crystal, the products with the reaction time of 10 min are mainly disk-like crystals and little spherical and cube-like crystals (5–15 μm). After 20 min small thorn spherical aggregates of rhombic crystal (with the diameter of 6–7 μm) and lamellar structured crystal (with the size of 3–4 μm) are formed. After 30 and 40 min flower-like crystals with the size of 4–5 μm and partly ellipsoidal crystals 5–6 μm in length and 2–3 μm in width and polyhedral structure of 3–4 μm are formed. After 50 min 4–5 μm sized spherical crystals assembled are obtained. 	–	Contributing for the understanding of biological mineralization.	Zhao et al., 2013

(Continued)

TABLE 1 | Continued

Objectives	Methodology	Experimental conditions	Studied variables	Physicochemical properties	Biological properties	Applications	References
Obtain HAp microspheres using CaCO ₃ as the template under the assistance of microwave irradiation.	Sericin = 2 g/L, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China). Precipitation method: 1°) Synthesis of CaCO ₃ particles through a co-precipitation method: - [CaCl ₂] (0.2 M) + sericin; 1°) Synthesis of HAp particles: Addition of [Na ₂ HPO ₄] (0.2 M).	1°) Mixture was stirred for 50 min; T = 25°C and pH = 7; 2°) Mixture was stirred for 180 min; T = 40°C; pH = 10.	Ca and P ratios of 1:0.15, 1:0.3, 1:0.45, 1:2.5; Mineralization time = 1, 3, 5, 10, 15 min; Microwave powers = 160, 320, 480, 640, and 800 W.	- Weight percentages of HAp in the composites are 19, 25, 40, 45, and 56% corresponding to the microwave power values of 160, 320, 480, 640, and 800 W, respectively; - With the increase of Ca/P ratio in the reaction solution from 1:0.15, 1:0.30, 1:0.45, 1:2.5 to 1:7, the content of HAp in the composite increases from 0.8, 1.4, 12.3, 50.0 to 99.0%; - The composite with higher HAp content showed a slower degradation speed; - The average diameter of the spherical particles is about 6.7 μm, which is close to the size of CaCO ₃ template.	-	Present the potential of sericin/HAp composites in the field of bone tissue engineering (for bone repair and regeneration).	Yang et al., 2016
Obtain HAp using spherical CaCO ₃ fabricated in the presence of sericin.	Sericin (g/L) = 2, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China); 1°) Synthesis of CaCO ₃ particles through a co-precipitation method: - [CaCl ₂] (0.2 M) + sericin; 2°) Addition of [Na ₂ HPO ₄] (0.2 M); 3°) Synthesis of HAp particles using a hydrothermal method: • CaCO ₃ powder was added into [Na ₂ HPO ₄] (0.1 M).	1°) Mixture was stirred for 30 min at room temperature; 2°) Mixture was stirred at 40°C for 180 min and dried at 60°C for 48 h. 2°) Mixture was maintained in na autoclave at 140°C for different times; Precipitate was dried at 60°C for 48 h.	Time in the autoclave = 2, 4, 8, and 16 h.	- The sericin/CaCO ₃ /HAp particles obtained are homogeneous microspheres with a size distribution from 3 to 10 μm; - Uniform porous HAp microspheres with high crystallinity were obtained; - Increasing time in the autoclave has little impact on morphology of sample but results in more rough surfaces.	- <i>In vitro</i> assays showed that the sericin/CaCO ₃ /HAp composites obtained are not toxic to MG-63 cells and have similar proliferation rates. Further, with the increase in HAp content higher cell viability was observed. - <i>In vivo</i> assays showed that sericin/CaCO ₃ /HAp composites are biodegradable and biocompatible when implanted into the groin subcutaneous tissue, using colloidal sodium alginate solution as a carrier. Additionally, sericin/CaCO ₃ have a higher degradation rate.	Present the potential of sericin/CaCO ₃ /HAp composites in the field of bone tissue engineering (for bone repair).	Zhong et al., 2016
Prepare sericin/monite composites using a cost-effective method.	- Sericin was obtained by extraction in boiling water. - Sericin was treated with ethanol, subjected to membrane dialysis and lyophilized. Co-precipitation method: [Na ₂ HPO ₄] (Sigma-Aldrich Chemicals Pvt Ltd; CAS Number 7558794) + sericin; [CaCl ₂ ·2H ₂ O] (1 M).	Mixture was stirred for 3 h.	-	- The particles obtained have a heterogeneous size distribution and different morphologies.	- <i>In vitro</i> assays showed that the sericin/monite composites do not elicit hemolysis of human blood. Further, the composites present cell viability and osteogenic properties and increase cell proliferation of MG-63 cells.	Present the potential of sericin/monite composites in the field of bone tissue engineering (as bone grafts).	Vedakumari et al., 2019

(Continued)

TABLE 1 | Continued

Objectives	Methodology	Experimental conditions	Studied variables	Physicochemical properties	Biological properties	Applications	References
Investigate the distribution and degradation of CaP/sericin nanoparticles <i>in vivo</i> .	<ul style="list-style-type: none"> - Sericin (mg/mL) = 10, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China). Co-precipitation method: [Na₂HPO₄] (30 mM); [CaCl₂] (50 mM) + sericin. 	pH = 9–10; T = 50°C.	–	<ul style="list-style-type: none"> - The sericin/CaP materials obtained adopt a spherical morphology with an average diameter of 80 nm; - The particles have a smooth surface and a low degree of crystallinity. 	<p><i>In vivo</i> tests evaluated the distribution, degradability and tumor targeting of the sericin/CaP composites;</p> <ul style="list-style-type: none"> - Distribution of sericin/CaP nanoparticles <i>in vivo</i> were mostly verified in the liver of mice; - The degradation time did not increase linearly with the amount of sericin/CaP nanoparticles injected <i>in vivo</i>; - Nanoparticles with an average size of 80 nm can achieve tumor localization at 7 days after the intravenous injection. 	Present the potential of sericin/CaP composites in the field of bone tissue engineering (for tumor therapy as a vehicle to deliver therapeutic medicine).	Zhao et al., 2017
Study the stability of sericin/CaP microcapsules for stability release.	<ul style="list-style-type: none"> Sericin (w/v%) = 0.2, purchased from Aotesi Biochemicals Co. Ltd (Huzhou, China). - Sericin microcapsules were obtained mixing the sericin solution with [CaCl₂] (30 mM) and by the posterior addition of amyllum (0.5%); Soaking method: - The sericin microcapsules were added to a supersaturated calcium phosphate solution [Ammonium phosphate solution (5 mM); calcium nitrate solution (10 mM); citric acid (2.5 mM)]. 	–	<p>Mineralization time: 3, 5, 6.5, and 12 h;</p> <p><i>In vitro</i> amyllum release at different pH values: 3, 5, 7, and 9;</p> <p>T = 37°C;</p> <p><i>In vitro</i> amyllum release at different ionic strengths: Sodium chloride solutions with 0, 0.1, 0.4, and 0.7 M.</p>	<ul style="list-style-type: none"> - An increase in the mineralization time led to an increase of the crystallinity and thickness of the microcapsules; Particles in the surface of the microparticles with a CaP salt-based composition and flake-like morphology after 12 h; - When the pH value was 3 the amyllum release rate was slow for the sericin microcapsules and the release amount increased gradually. After 100 h, the release rate decreased, and the release amount did not increase. pH 5 and 7 with similar behavior, characterized by a faster release rate. At pH 9, the composite became unstable; - The release rate was very fast when the concentration of NaCl in the controlled-releasing solution was 0.7 M at 37°C, being slower for other concentrations. Further, at lower pH values sericin/CaP microspheres release rate was rather slower when compared to sericin microspheres. 	<i>In vitro</i> assays showed the composites promote the viability and proliferation of HapG-2 cells, when compared to sericin microcapsules.	Present the potential of sericin/HAp composites in the field of bone tissue engineering (for drug delivery and encapsulating bioactive molecule systems).	Li et al., 2016
<i>In vivo</i> biodegradation evaluation of the α-TCP porous body coated with SS.	<ul style="list-style-type: none"> Sericin was extracted from raw silk fiber by an autoclave at 105°C for 60 min and stored at 4°C for 2 weeks. Soaking method: - A α-TCP block was soaked into a sericin solution. 	–	–	–	<i>In vivo</i> tests showed that after 4 weeks of implantation on bone defects made in rabbit tibiae and subcutaneous sites of rabbit backs, higher density of cortical bone was estimated for α-TCP coated with sericin than for mere α-TCP.	Present the potential of sericin/HAp composites in the field of bone tissue engineering (for bone repair as a biodegradable materials).	Takeuchi et al., 2005a

(Continued)

TABLE 1 | Continued

Objectives	Methodology	Experimental conditions	Studied variables	Physicochemical properties	Biological properties	Applications	References
Fabrication of hierarchical bio-hybrid architectures through a green and facile method.	Sericin (mg/mL) = 0.1, purchased from Wako Chemicals, Japan. Co-precipitation method: [CaCl ₂] (100 mM) + sericin PBS.	T = 25°C; Mineralization time: 24 h.	-	<ul style="list-style-type: none"> - Porous, uniformly grown flower-like composites were obtained (average size 2–5 μm); - The composites have highly crystalline characteristics when compared to pure sericin; - These composites have a large surface area and show excellent adsorption activity for the toxic heavy metal ions of Lead (II), cadmium (II), and mercury (II) from wastewater. 	-	Present the potential of sericin/HAp composites for multimodal applications such as adsorption of toxic heavy metals and hazardous dye from wastewater.	Koley et al., 2016

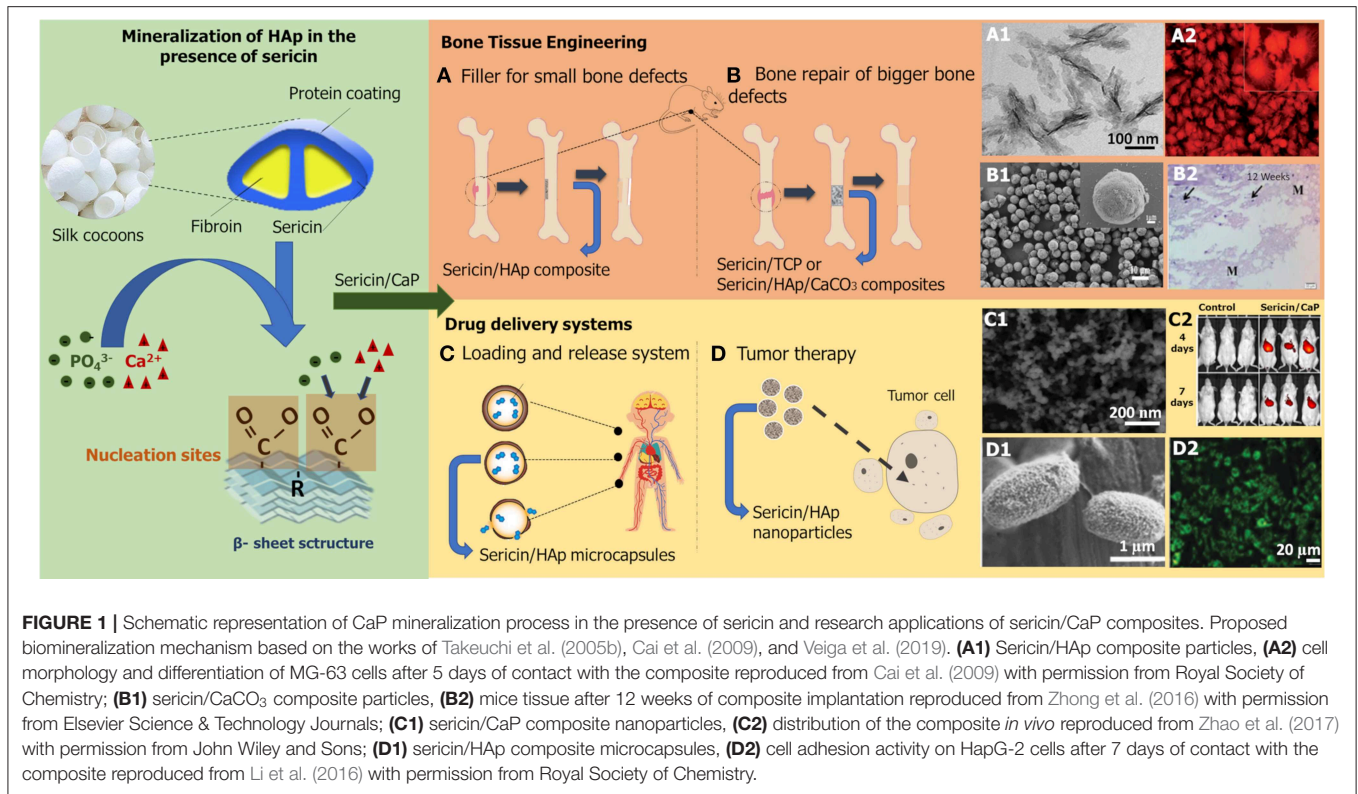
The objectives and suggested applications of the reviewed papers, as well as the methodology, experimental conditions, studied variables, and main results are highlighted.

Therefore, sericin/CaP biomaterials can better perform as, for instances, a particulate system for bone filling rather than as a 3D regenerative material (Jones, 2013). Accordingly, studies focus on the synthesis of sericin/CaP nanoparticles (Cai et al., 2009), and films (Jones, 2013; Zhang et al., 2014). Sericin/CaP nanoparticles have been described as suitable for bone filling applications due to their biocompatibility, biodegradation, strong ability to promote cell differentiation and proliferation and possibility to deliver therapeutic medicine. Sericin/CaP composite films have also potential in bone-related applications, being biocompatible and promoting cell viability. Further, both particles and films can be used to for fundamental studies of cell–matrix interactions (Table 1).

HAp is a relatively inert material that is retained *in vivo* for prolonged periods of time due to its slow reabsorption rate (Campana et al., 2014). Thus, sericin/HAp composites have potential as coating in orthopedic or dental implants. This is in agreement with the studies of Klein et al. (1991) and Dhert et al. (1993) where it was found that, when used as a coating material, HAp exhibits excellent bone contact with implanted titanium prosthesis. By comparison, tricalcium phosphate (TCP) materials give rise to more bone remodeling lacunae along the implant surface (Klein et al., 1991). Additionally, it has also been reported that the use of sericin can improve the osseointegration and osteoconduction of orthopedic titanium implants (Nayak et al., 2013), and that sericin/HAp composites have excellent biocompatibility and cell viability (Yang et al., 2014a; Zhang et al., 2014).

In contrast to HAp, TCP-based scaffolds are resorbable, making TCP composites specially suitable for the treatment of small bone defects (Bohner, 2000). In the study of Chazono et al., a TCP-based material led to complete new bone formation after 13–20 weeks of implantation (Chazono et al., 2004). Also, TCP appears to have superior osteoconductivity and bone remodeling, when compared to HAp (Ogose et al., 2005). The co-precipitation of sericin with TCP is demonstrated to decrease the degradation rate and improve the biological performance. According to the results of Takeuchi et al. (2005a), sericin/α-TCP materials show slower degradation in bone defect of rabbits, when compared to pure α-TCP. Other sericin/CaP composites, such as sericin/HAp/calcium carbonate (CaCO₃), have also showed controllable degradability properties (Zhao et al., 2013; Yang et al., 2016). In published works, the formation of sericin/HAp composites, was achieved through the conversion of sericin/CaCO₃ particles using the assistance of microwave irradiation (Yang et al., 2016) or via a hydrothermal method (Zhong et al., 2016). A higher HAp content was also associated with a slower degradation rate, being therefore appropriate for bone regeneration.

In vivo experiments showed that dicalcium phosphate anhydrous (DCPA), also known as monetite, can resorb faster than most CaP, supporting implant replacement by newly formed tissue (Tamimi et al., 2012). Thus, the sericin/monetite synthesized by Vedakumari et al. (2019) has potential as biodegradable materials.



Drug Delivery Systems

Sericin/CaPs materials have also been proposed as drug delivery systems. Due to their biodegradability and hydrophilicity sericin-based materials may be used as a vehicle to deliver therapeutic medicine (Wang et al., 2015). Ideally, these systems should have controlled degradation, enable easy binding of other molecules and have pH responsiveness (Kunz et al., 2016). Sericin/CaP composites can be designed as microsystems with a good loading and release capability for bioactive molecules to be applied in TE or as an anti-tumor therapeutic system (Table 1). The drug release potential of sericin/CaP microcapsules was studied in a paper by Li et al. (2016), by incubating sericin microcapsules with a supersaturated calcium phosphate solution containing citric acid. The CaP shell protected the inner sericin structure, and consequently the stored bioactive molecules until a pH change occurs. Not only did the capsules showed an improved stability and a longer storage time capability, but also increased cell viability.

In the work of Zhao et al. (2017), sericin/CaP nanoparticles were developed and injected into mice by intravenous injection, to allow particle distribution through the whole body. Analysis of the *ex vivo* organs showed that 80 nm spherical sericin/CaP nanoparticles are almost completely distributed in the liver cells of mice ($\approx 90\%$) and can be biodegraded within 2 weeks. However, the degradation rate is not proportional to the amount of composites injected, as 100 and 200 μg of nanoparticles were degraded after 48 h and 13 days, respectively. The targeting percentage was 30% at 7 days post-injection. As no particles were found in other organs, it was concluded that these composites can target specific solid tumors *in vivo*.

Nowadays, nanoparticle pharmaceutical carriers are probably amongst the most studied sericin/CaP composites, making these very interesting composites in this field. The large surface area/volume ratio provides the capability to load large amounts of biomolecules and the ability to go across the cellular membrane (Jia et al., 2013).

Other Applications

As the study of sericin/CaP composite materials is relatively recent, there are still several areas to be explored, taking advantage of the individual properties of sericin and CaP. For instance, calcium has an important role in maintaining homeostasis in mammalian skin, promoting wound healing (Lansdown, 2002; Kawai et al., 2011). In particular calcium-based nanoparticles have shown to have therapeutic benefits when used in cutaneous wound repair (Kawai et al., 2011). On the other hand, sericin has high antioxidant potential (Prasong, 2011; Kumar and Mandal, 2017), antibacterial activity (Ramesan et al., 2016), mitogenic effect on cells (Terada et al., 2005), potential to inhibit inflammation (Aramwit et al., 2012a), and ability to retain hydration (Padamwar et al., 2005). In the study of Aramwit et al., a sericin-based therapeutic cream formulation improved healing by promoting collagen production and rapid re-epithelialization in rat skin wounds (Aramwit et al., 2009, 2013). Both two-dimensional (films) and three-dimensional (scaffolds) sericin matrices, obtained using different blending methods, have been reported for skin tissue repair (Nayak et al., 2012; Lamboni et al., 2015; Karahaliloglu et al., 2017). Therefore, a sericin/CaP biomaterial could significantly accelerate and improves wound healing and soft tissue replacement. While sericin has been used

in many cosmetic products over the years, CaP-based materials are only recently being explored in this area. CaP particles can be used as a bone filler in esthetical treatments for diminishing wrinkles by stimulating conjunctive tissue formation (Lopes et al., 2009). Due to its affinity for biopolymers, crystallites of HAP in the dermis can promote the self-assembly of collagen fibers, smoothing fine lines and even deeper wrinkles (Bell, 2012; Gupta et al., 2019). CaP-based sunscreens have also been studied due to their biocompatible nature that does not generate allergies and other skin irritations, contrary to many organic sunscreens (de Araujo et al., 2010). These properties coupled with sericin strong suppressing activity against UVB-induced acute damage and tumor promotion (Zhaorigetu et al., 2003; Kumar et al., 2018), anti-wrinkle effect and anti-tyrosinase characteristics (Chlapanidas et al., 2013; Kumar and Mandal, 2019) justify the development of sericin/CaP biomaterials for the cosmetic industry.

Different CaP phases are used in food applications, as a supplement to increase calcium and phosphorus intake or as food additives to thicken and stabilize firm foods (Eliaz and Metoki, 2017). Phosphate additives are widely used in processed foods as preservatives, acidifying agents, acidity buffers, and emulsifying agents. Despite their applications, the use of non-naturally occurring phosphates in food can negatively affect the general health of the consumer. Synthetically produced phosphates are effectively absorbed in the gastrointestinal tract, whereas in “organic” phosphates, the absorption percentage ranges between 40 and 60%. This can lead to calcification in blood vessels and organs, being also potentially harmful for patients with chronic kidney disease (Sherman, 2007; Ritz et al., 2012). Studies show that sericin can be used to prevent functional gastrointestinal disorders, improving ion absorption and consequent bioavailability of these elements (zinc, iron, magnesium, and calcium ions) and improving constipation (Sasaki et al., 2000a,b). These characteristics can inspire the synthesis of sericin/CaP materials also for the food industry.

Recent applications of sericin/CaP composites include the removal of micro-pollutants from wastewater. In the work of Koley et al. (2016), sericin/HAP materials were used to purify water, exhibiting excellent adsorption of toxic heavy metal ions. While HAP has stability, sericin is a water-soluble material, capable of forming hydrogen bonds and with strong metal binding properties (Khosa et al., 2014).

RECENT ADVANCES

Despite their properties of interest and numerous applications, sericin/CaP composites still have disadvantages to circumvent such as poor mechanical resistance under complex stress states. CaPs and pure sericin have a low mechanical profile when compared to bone tissue, which limits the application of sericin/CaP composites as 3D biomaterials for load-bearing applications (Kunz et al., 2016; Canillas et al., 2017). Of the reviewed papers, only Zhang et al. (2014) reported the mechanical properties of sericin/HAP composites, obtained by mineralization of a flexible ethanol-treated sericin film. Other

explored alternatives to enhance these characteristics include the integration of other components in the composite. Adding a structural organic component such as collagen (Griffanti et al., 2019; Jang et al., 2019), chitosan (Chen et al., 2015), and alginate (Zhang et al., 2015); or a synthetic polymer such as polymethyl methacrylate (PMMA) (Tontowi et al., 2017) are examples of some of the most recent approaches. Structural integrity of the constructs can also be obtained by cross-linking the components with glutaraldehyde (GTA) (Bhowmick et al., 2016).

CONCLUSIONS

Up to recently, silk sericin was traditionally disposed during silk processing. Nowadays, there has been a growing interest in its recovery due to both its beneficial properties and environmental impact caused by its generation. Works on sericin/CaP composites focus mainly on understanding the biomineralization process, through the study of the effect of sericin in HAP nucleation. Other studies dedicate to the synthesis of these biomaterials for the substitution and regeneration of hard tissues and to the development of new drug delivery systems. Apart from the biomedical field, there are other areas where these composites have a lot of potential, such as in the cosmetic, food and environmental sector.

In the last decade, the number of published papers on sericin/CaP composites has increased substantially. Thus, in the near future, it is clear that the integration of silk sericin in CaP biomaterials will result in advanced composite systems with evident benefits on several research fields, particularly in biomedical and pharmaceutical-related applications.

AUTHOR CONTRIBUTIONS

All authors contributed to the analysis and writing of the manuscript, and approved it for publication.

FUNDING

This work was financially supported by: Base Funding – UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE – funded by national funds through the FCT/MCTES (PIDDAC). The authors also acknowledge Portuguese National Funds from FCT—Fundação para a Ciência e a Tecnologia through project UID/Multi/04044/2019. This work was supported by project Biotherapies-Bioengineered Therapies for Infectious Diseases and Tissue Regeneration and Interreg V-A POCTEP Programme through FEDER funds from the European Union [0245_IBEROS_1_E] C. A.

ACKNOWLEDGMENTS

The authors also extend their deep appreciation to Department of Chemical Engineering at Faculty of Engineering of the University of Porto, and Centre for Biotechnology and Fine Chemistry at School of Biotechnology of the Portuguese Catholic University.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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