Tissue engineering using scaffolds for bone reconstruction: a review of sol-gel silica materials for bone morphogenetic proteins (BMP) encapsulation and release

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Abstract

This review focused on sol-gel silica materials for surface modification of scaffolds for bone reconstruction. These silica materials can be tuned to adsorb and release specific biomolecules involved in bone reconstruction. This tuning can be possible by changing the synthesis parameters as the pH, the solvent or the presence of surfactant or by modifying its surface properties by including functional groups. It results in silica materials with very specific pore structure as hexagonal, cubic or lamellar.

The grafted biomolecule studied in this review is the bone morphogenetic protein (BMP). Two methods have been highlighted in the literature for its encapsulation on silica: (i) the impregnation of already synthesized silica gels in a protein solution and (ii) the direct encapsulation of the protein during the gel formation. Both methods succeeded for adsorption and release but a good balance between these two phenomena is necessary to promote bone reconstruction. The two main parameters that will impact the protein loading and release are the texture and the surface chemistry of silica.

Finally, some strategies for silica deposition on scaffolds are reviewed: the coating of the substrate with a silica film and the deposition of a dispersion of silica particles contained in another matrix. The second option seems more feasible as the deposition of a silica film requires to synthesize and process the silica gel simultaneously.

Keywords

Bone reconstruction, sol-gel process, silica, biomaterials, biomolecule grafting

1. Introduction

These past decades, the interest for medical devices for bone reconstruction has been constantly rising. The worldwide market for bone grafts and substitutes was worth $2,652 million in 2020 [1]. This represents 4 million surgical operations involving bone grafting or bone graft substitutes per year, making bone the second most grafted organ after blood transfusion [2]. In the next year, the interest for bone reconstruction is predicted to continuously grow, reaching $3,362 million by 2028, thanks to the rising number of orthopedic surgery (ageing of the population, increase of need of healthcare in developing countries) [3].

In most cases, bone is able to regenerate itself without any scar [4,5]. However, in case of large fracture defects (*i.e.* gaps beyond two and a half times the bone radius), bone cannot reconstruct itself and

assistance is needed in order to recover the complete structure and function of the native bone [6]. In numerous cases, an artificial substitute or an organ transplant can be used in order to repair the broken bone. If these approaches are successful in several clinical situations, they present important drawbacks like a limited lifetime or a lack of donors [7,8]. In order to overcome these problems, a new approach has been recently developed, *i.e*. tissue engineering. Tissue engineering aims at the reconstruction of human tissue by using biocompatible biodegradable porous materials, called scaffolds, as templates to support and control the spatial organization of the cells [9–11]. Among the different materials proposed for this purpose, calcium phosphate (CaP) ceramics have a great potential. However, the suffer from a lack of cell differentiation, bone production, and bone integration at their surface [12,13]. An interesting solution to promote bone regeneration is the surface modification of these biodegradable scaffolds to incorporate growth factors that could be delivered locally in a sustained manner.

In this context, silica gels have been specifically developed for the encapsulation and controlled release of different biomolecules [14–19]. They present the advantage of allowing the absorption of a high amount of biomolecules thanks to their large specific surface area [20]. Moreover, the morphology and the surface chemistry of the pores can be tuned depending on the synthesis conditions (e.g. pH, solvent, processing conditions, grafting of functional groups) to modulate the interactions between the pores and the protein (i.e. electrostatic forces, hydrogen bonding, van der Waals forces and covalent interactions) and to subsequently regulate the encapsulation/release process [21–23]. Additionally, silica is biocompatible and resorbable [24–26]. According to this approach, silica loaded with active macromolecules can be deposited at the surface of bone scaffold in order to improve bone repair process.

This work will review the tissue engineering approach to bone reconstruction and, more specifically, the use of silica material as biocompatible matrix to graft bone morphogenetic proteins (BMP). After a first part on bone reconstruction and modelling, the use of scaffold for bone reconstruction will be presented. Then, the surface modification of these scaffolds with silica will be reviewed with a focus on the encapsulation of BMP. This review highlights the main factors influencing the encapsulation and release of proteins in silica in order to find the link between them, which is not usually shown in the literature. Sol-gel synthesis of silica will be of great interest in this review due to the mild conditions of production and the easy tuning of the silica material obtained with sol-gel process.

2. Bone remodeling and reconstruction

2.1. Bone remodeling

Bone is a highly dynamic tissue that undergoes continuous remodeling [27]. Bone remodeling is essential to maintain bone volume, integrity, and strength, to adapt bone structure to changes in mechanical stresses, and to regulate mineral concentration in the body [27,28]. It is estimated that 2 to 5 % of cortical bone is being remodeled every year [29]. In homeostatic equilibrium, bone resorption and bone formation occur at the same rate so that the bone volume is maintained [30]. However, in the case of some diseases, one can be increased compared to the other, resulting in a decrease (*e.g*. osteoporosis) or an increase (*e.g*. osteopetrosis) in bone mass [28,31]. A resorption cycle occurs in four main stages (*i.e*. activation, resorption, reversal, and formation) and takes 5-6 months [32,33].

During the activation phase, a remodeling site is recognized in a random manner or on the mechanical request of osteocytes [30,33,34]. Upon activation, bone lining cells retract from the remodeling site and osteoclasts are recruited via the release of cytokines [35]. The resorption phase then occurs. Osteoblasts first release hydrogen ions via H^+-ATPase proton pumps and chloride channels located in the ruffled border into the Howship's lacuna to lower the pH and dissolve hydroxyapatite crystals [36,37]. After solubilization of the mineral phase, several proteolytic enzymes such as tartrate-resistant acid phosphatase, cathepsin K, and matrix metalloproteinases are secreted via the ruffled border into the resorption lacuna to degrade the organic bone matrix [33,38–40]. After resorption, the reversal phase

takes place. During this phase, mononuclear cells present at the bone surface further degrade collagen, deposit proteoglycans and release factors to recruit preosteoblasts that will proliferate and differentiate [30,33,41]. The next phase consists in bone formation by osteoblasts. Osteoblasts will first deposit the organic matrix, called osteoid [30,33,42]. Osteoblasts release then vesicles that concentrate calcium and phosphate [33,43]. This leads to the precipitation of hydroxyapatite between the collagen fibers that will act as nucleation agent due to their conformation [30,44]. Finally, when bone remodeling is complete, bone lining cells cover the surface of bone [32].

2.2. Bone reconstruction

During the reconstruction process, several cell types colonize bone defects. The main process by which new bone is formed, is called secondary healing [45–47]. This mechanism leads to the formation of a totally self-regenerated bone with structural and mechanical properties similar to the preexisted bone tissue and without the production of scar [48]. Secondary healing involves three main phases: inflammation, repair, and remodeling (**Erreur ! Source du renvoi introuvable.**) [47,49,50]. Immediately after the fracture and the severing of blood vessels, a hematoma is formed as a consequence of blood rushing to the fracture site [51]. Biochemical factors are also released, which attracts mesenchymal stem cells (MSC) from the periosteum and soft tissues around the fracture sites [47,49]. This is the inflammatory phase. The repair phase is divided into the soft callus and the hard callus phases [49–51]. During the soft callus phase, MSC differentiate into different cell types, which are selected in function of the local conditions, notably the nature of growth factors and the oxygen concentration. Far away from the gap and near the cortex, MSC differentiate into osteoblasts that synthesize woven bone. In the central region, MSC differentiate into chondrocytes able to produce cartilage, which mechanically stabilizes the fracture. Eventually, the fracture gap is bridged by cartilage and woven bone. During the hard callus phase, chondrocytes become hypertrophic and cartilage is mineralized, leading to the resorption of the soft callus and its replacement by woven bone. As cartilage is being calcified, the mechanical properties and the rigidity of the fracture site increase, allowing weight bearing. Meanwhile, bone is also vascularized. During the remodeling phase, woven bone is resorbed by osteoclasts, while osteoblasts progressively build the lamellar bone [47,51]. Ultimately, bone is regenerated and regains its original structural and mechanical properties [47,49,50].

3. Tissue engineering

3.1. Scaffolds

The self-repairing ability of bones presents some limit capacity explaining that for large fracture defects (*i.e.* gaps beyond two and a half times the bone radius), assistance is needed in order to recover the complete structure and function of the native bone [52]. In numerous cases, an artificial substitute has to be implanted in order to replace the function of the defective tissue [53–55]. This method raises several difficulties like the risk of infections, a short lifetime, and biomechanical problems (*e.g.* fracture, loosening, wear, corrosion) [56–58]. Besides this technique, a frequent alternative is organ transplant [48,59]. However, this approach presents important drawbacks, such as the lack of donors, disease transfer, the presence of several surgery sites, and the eventual rejection by the recipient [60–62]. In order to overcome these problems, a new technology has been recently developed, tissue engineering. During the past few years, tissue engineering has become one of the most promising techniques to maintain, improve, or reconstruct human tissue, even complete human organs [63–65]. Tissue engineering avoids long-term biocompatibility problem of synthetic implants which can enhance infection, biomechanical problems (*e.g.* break, loosening, corrosion), or inflammatory response. In addition to the injection of free stem cells, tissue engineering also uses biomaterials, called scaffolds. Scaffolds are temporary porous three-dimensional matrices that serve as support and template for the

development of cells [63,66]. During the reconstruction process, scaffolds are degraded inside the body and replaced by a newly formed tissue. At the end, only reconstructed or repaired tissue is present.

To guarantee an optimal bone regeneration, scaffolds have to satisfy very strict and specific requirements [67–69]. First, scaffolds have to be biocompatible in order to allow cell growth and proliferation. To satisfy this former condition, scaffolds must present an interconnected porosity in order to promote cell colonization as well as the diffusion of nutriments, metabolites, and gases [70]. The degradation rate of the material has to be adjusted according to a kinetics compatible with bone repair (typically between 2 and 6 months), with products of degradation that can be metabolized or cleared rapidly from the body. Mechanical properties of implants have to closely approach bone mechanical properties in order to guarantee organ functions during the reconstruction period [69]. Finally, the scaffold must be bioactive (**Erreur ! Source du renvoi introuvable.**) by promoting the osteoconduction (*i.e.* infiltration of cells from the host body through the pores of the matrix), the osteoinduction (*i.e.* in the case of the use of stem cells, differentiation of stem cells into bone cells), the osteogenesis (*i.e.* production of extracellular matrix by the bone cells), and the osseointegration (*i.e.* structural and functional connection of the scaffold with the bone).

4. Surface modification

4.1. Drug delivery

Various materials have been proposed for the conception of scaffolds. Several ones are frequently used nowadays, like metals, polymers, ceramics, bioglasses, and composites [71–80]. Yet, one main problem of these materials is their lack of bioactivity, notably in terms of osteogenesis and osseointegration [81–85]. In recent years, considerable attention has been given to the administration of therapeutic biomolecules to promote tissue regeneration [85–87].

The ideal way for the administration of these molecules is the encapsulation of biomolecules and their subsequent controlled release in the body (**Erreur ! Source du renvoi introuvable.**). Biomolecules are encapsulated in a porous material that is deposited at the surface of the pores of the scaffold. Once inside the body, the biomolecules can diffuse out of the scaffold and trigger specific messages in the cells present in the surrounding tissue. This technology, called drug delivery, improves the efficiency of current delivery methods (*e.g.* oral and blood delivery) by targeting a specific treatment zone and controlling the release of biomolecules (*e.g.* proteins, hormones, antibiotics) on the long term [88,89]. Accordingly, it is possible to deliver the drug or the biomolecule contained inside the pores of a matrix to the desired specific location inside the body, considerably increasing the success rate by reducing toxicity. Moreover, the biomolecule can be released in a sustained way on a long period (from minutes to months according to the application) [90,91].

4.2. Bone morphogenetic protein (BMP)

Among the biomolecules considered for bone reconstruction, bone morphogenetic proteins (BMP) appear as good candidates [92–94]. BMP are a family of cytokines part of the transforming growth factor-β superfamily that have been shown to induce significant bone formation via the regulation of cell adhesion, proliferation, differentiation, and apoptosis in bone tissue. Specifically, BMP-2 is able to promote the bone differentiation of stem cells and to accelerate the fracture repair via the recruitment of osteogenic progenitor cells [95–97]. Clinically, two BMP have been approved by the Food and Drug Administration for the treatment of bone fractures: BMP-2 and BMP-7 [98,99]. Yet, the release of BMP from the matrix must be carefully controlled to avoid an excessively high dose of growth factors, which would lead to side effects, such as undesirable growth of tumors or neovascularization of non-targeted tissues [100].

Another drawback of these proteins is their prohibitive price of BMP (*i.e.* close to 600 €/ 10 µg) [101]. For this reason, in the literature, a model protein is often used: the Soybean Trypsin Inhibitor (STI) [102–104]. STI is selected for its size, molecular weight, point of zero charge, hydrophobic properties, and release kinetics, which are close to the BMP ones (Table 1) [24,105,106].

Table 1 – Properties of STI and BMP-2 [103,107–111].

	STI	**BMP-2**
Molecular weight (g/mol)	21,000	26,000
Size (nm)	4.5 x 4.2 x 4	7 x 3.5 x 3
Point of zero charge (pH unit)	4.5	4.8-5.1

4.3. Silica gels

Several materials have already been proposed for the sustained delivery of BMP, such as polylactide-co-glycolide [112–114], agarose [115], titania [116], and lipids [117]. Among them, silica gels represent good candidates. Sol-gel silica is an amorphous porous materials formed by an interconnected network of silicon atoms presenting a large surface area [103,118,119]. This characteristic makes them able to absorb a large quantity of biomolecules [120]. Moreover, a large control on the morphology and the surface chemistry of the pores can be obtained depending on the synthesis conditions (*e.g.* pH, solvent, processing conditions) [22,121]. These properties will affect the interaction of the proteins with the pores (*i.e.* electrostatic forces, hydrogen bonding, van der Waals forces, covalent interactions) and in turn the encapsulation/release process. For example, different functional groups can be grafted at the surface of the pores to modulate the charges or the hydrophilic/hydrophobic balance of the materials [122–125]. Another advantage is the synthesis conditions, which are not detrimental for biomolecules (mild temperature and pressure conditions) [103,118,119]. Studies have even demonstrated that enzymes encapsulated in silica retained their structural integrity and their biological activity [126]. From a processing perspective, different forms of silica gels can be obtained, including monoliths, films, and (micro- and nano-)particles [103,121,127]. From a biocompatibility point of view, silica is resorbable and non-toxic ("Generally Recognized As Safe" by the US Food and Drug Administration) [25,26,103,128]. Other advantages of silica include negligible swelling and mechanical stability [129,130].

Silica gels can be synthetized via the sol-gel process. The sol-gel process consists in the formation of a suspension of solid particles in a liquid, called sol, followed by the formation of a tridimensional network of condensed particles, called gel [131–133]. Sol-gel syntheses are carried out in mild conditions of temperature and pressure. In the present study, sol-gel process involves the hydrolysis and condensation of a silicon alkoxides ($Si(OR)_4$) in an alcoholic medium. In the hydrolysis step (Equation 1), an alkoxy group (≡Si-OR) is replaced by a silanol group (≡Si-OH) by nucleophilic substitution [134–136]. In the condensation step, the formed silanol group can react with an alkoxy group (Equation 2) or another silanol group (Equation 3) to form a siloxane bond (≡Si-O-Si≡). These reactions are reversible.

$$\equiv Si\text{-}OR + H_2O \leftrightarrow \equiv Si\text{-}OH + ROH \quad (1)$$

$$\equiv Si\text{-}OR + OH\text{-}Si\equiv \leftrightarrow \equiv Si\text{-}O\text{-}Si\equiv + ROH \quad (2)$$

$$\equiv Si\text{-}OH + OH\text{-}Si\equiv \leftrightarrow \equiv Si\text{-}O\text{-}Si\equiv + H_2O \quad (3)$$

4.4. Structured mesoporous silica

Structured mesoporous silica (SMS) is a porous material, with pore size ranging between 2 and 50 nm, presenting a well-defined periodic geometry. Structuring agents like surfactants can be used as templates to obtain this organized geometry (**Erreur ! Source du renvoi introuvable.**) [137–139]. Surfactants are amphiphilic molecules (*i.e.* presenting a hydrophobic and a hydrophilic part), which form micelles (*i.e.* an aggregate of molecules in a colloidal solution) above a certain concentration, called critical micelle concentration. At higher surfactant concentration, micelles organize themselves in the solution and assembly in liquid crystal arrays that are organized at the mesoscopic scale. After silica precursor addition, these micelles act as a template for the formation of the silica. At the end of the synthesis, the surfactant is removed by solvent extraction or calcination, leaving the inorganic material with the desired geometry [140–143].

The nature of the surfactants plays a key role in the formation of the structure and the pore size of SMS [144–146]. Indeed, the surfactant type (*e.g.* cationic, anionic, non-ionic, zwitterionic) determines the type of interactions between the chemical reagents in solution and the surfactant, leading to specific mesostructure [143,147–150]. Moreover, the hydrophobic/hydrophilic character as well as the chains length can be tuned to obtain different mesostructures and pores sizes. For a same surfactant, its concentration can also greatly affect the mesostructure [147,148,151]. The pH is another essential parameter to control the interactions between the surfactant and the surrounding molecules through the charges of these different chemical species [143,152]. By adjusting these parameters, a wide range of structures has been obtained (Table 2). For example, longitudinal pores presenting a hexagonal cross-section (*e.g.* MCM-41, SBA-15) or 3D systems with intersections of the longitudinal pores in the three directions can be observed (*e.g.* MCM-48, SBA-16). To further increase the silica pore size, swelling agents (also called micelle expanders) such as alcohol, aliphatic hydrocarbons or aromatic hydrocarbons can be added during the synthesis [153–158]. These molecules can dissolve inside the micelles, which causes a swelling of the later and consequently an increase in the pore size. Nevertheless, these additives can reduce the degree of organization of the material or can change its structure [159].

Table 2 – List of some SMS structures [143,147,160–162].

SMS type	Pore symmetry	Surfactants	Pore size (nm)
MCM-41	2D hexagonal *P6mm*	CTAB, C_nTMA^+ (n = 12-18)	2-10
MCM-48	3D cubic *Ia3d*	CTAB, C_nTMA^+ (n = 14-18)	2-5
MCM-50	Lamellar *p2*	CTAB	2-5
SBA-15	2D hexagonal *p6mm*	B50-1500, P123, P85, P65, Brij97	5-30
SBA-16	Cubic *Im3m*	F127, F108, F98	5-15
FDU-12	Cubic *Fm3m*	F127	4-27
KIT-5	Hexagonal *P6m*	F127	9.3

Note: MCM = Mobil Crystalline Materials; SBA = Santa Barbara Amorphous; FDU = Fudan University; KIT = Korea Advanced Institute of Science and Technology.

SMS such as MCM-41, MCM-48, SBA-15, and SBA-16 have been widely used for the optimization of drug delivery [163–170]. Their well-defined textural properties (*e.g.* ordered pores and homogenous pore size distribution) allow a large control of the diffusion of the biomolecule inside the pores and thus of the biomolecule release outside of the materials. The release can be further regulated by the use of gates or caps [147,162,171,172]. This technique consists in grafting specific responsive molecules at the pore opening after loading the biomolecule, therefore preventing the release of the cargo. Once inside the body, the gates will open upon a specific stimulus and allow the release of the loaded biomolecules. These caps can notably be by a change in pH [173–176], a change in temperature [177–179], the addition

of reducing/oxidizing agents [180–182], by enzyme cleavage [183–185], by light irradiation [186–190], by exposition to magnetic fields [191,192] or a combination of these stimuli [193].

4.5. Encapsulation and release of BMP

4.5.1. Encapsulation and release of BMP

The encapsulation of BMP and STI inside silica gels has already been studied, as shown in Table 3. Due to the versatility of the sol-gel method, silica gels have been processed under different forms such as particles, films, and even scaffolds. These studies have shown that immobilization of these proteins in silica and their subsequent release over several days and even weeks is feasible. A fast release in the first hours or days, called burst, was sometimes observed. Yet, giving the label "burst" remained quite subjective and depends mainly on the authors' choice as there is not a clear definition in terms of quantity delivered or duration. A large majority of the studies focused on non-modified silica. Only a minority tried to assess the influence of the silica structure and composition on the protein encapsulation and release.

The direct comparison of the different studies is quite complicated. First, the results were expressed in variable ways, *e.g*. loading in %, mass of protein per surface area of silica, mass of protein per mass of silica, mass of protein per sample, while the studies did not provide enough information to convert this data. Another limitation was the use of different encapsulation and release protocols in terms of medium, concentration, duration, encapsulation method. Yet, these parameters can greatly influence the obtained results. Finally, even if the materials were characterized in terms of composition and porosity, most studies did not directly present the link between the textural properties and surface chemistry of silica, and the release kinetics.

It is also important to mention that only one study analyze the remaining activity of the protein after encapsulation and release, which is an essential factor to assess the possible *in vitro* and *in vivo* efficiency. A material providing an ideal release kinetics with a large deactivation of the protein is useless.

Despite these limitations, some interesting conclusions could be drawn, notably the influence on the encapsulation method (section 4.5.2.), of the physicochemical properties of silica (section 4.5.3.)

Table 3 – Encapsulation of BMP-like proteins in silica gels.

Silica type	**Encaps. tech.**	**Prot.**	**Encapsulation / release results**	**Ref.**
PEG coated MCM-41 type particles	Imp.	STI	L: 16 wt.%; R: continuous over 24 days (total released: 60 %); A: n.a.	[194]
Microporous films on titanium	Imp.	BMP-2	L: 5.7 ng/mm²*; R: continuous over 9 days (total released: 2 %*), slower than on uncoated titania (total released: 4 %*); A: n.a	[195,196]
Nanotubes	Imp.	BMP-2	L: 86 %; R: continuous over 13 days; A: n.a	[197]
pH-responsive MCM-41 type particles	Imp.	BMP-2	L: 248 µg/g; R: burst over 4 h then slower until 24 h (total released: 90 %); A: n.a	[198]

$CaSO_4$ cement/SBA-15 type particles	Imp.	BMP-2	L: 50 µg/sample; R: burst over 24 h (≈ 35 %) then continuous over 7 days (total released ≈ 70 %); A: n.a	[199]
Scaffold with a 3D cubic mesoporous structure	Imp.	BMP-2	L: 1, 5, 50 µg/sample (2-4 mg/g*); R: burst over 24 h (20 %) followed by continuous over 28 days (total released: 80 %); A: conformation change of 3.5 %	[200]
Amino-modified structured and non-structured silica films on glass	Imp.	BMP-2	L: 0 (no modification, regardless of the structure), 15 (unstructured), 12 (structured) ng/cm²; R: no release up to 2 days, 5 % after 5 days; A: n.a	[201]
Xerogel particles containing Ca, PO_4, and/or Mg	*In situ*	BMP	L: n.a.; R: gradual release (no quantitative data) ; A: n.a	[202]
Xerogel particles	*In situ*	TGF-β	L: 0.5-1 µg/sample; R: burst over 24 h (total released ≈ 1 %); A: n.a	[203]
Xerogel films on titanium	*In situ*	STI	L: 2.5 mg/g*; R: fast over 5 days (≈ 50 %) then continuous over 30 days (total released: ≈ 80 %) ; A: no loss of activity	[103]
Xerogel particles	*In situ*	STI	L: 0.6, 1.6, 3.3 mg/g; R: dose- and time-dependent, continuous over 9 weeks (total released: 21, 43, 32 %); A: n.a	[111]
Structured, unstructured, and organosilica particles	*In situ*	STI	L: 19, 38, 21 mg/g; R: burst over 24 h + continuous over 45 days (total released: 0-70 %).	[204]
Ethyleneamino-modified and non-modified particles	Imp. / *in situ*	STI	L: 14-23 mg/g; R: burst over 24 h + continuous over 80 days (total released: 20-90 %); A: 0-90 % of remaining activity.	[205]
Ethyleneamino-modified, amino-, and phenyl-modified particles	Imp. / *in situ*	STI	L: 8, 7, 58 mg/g; R: burst over 24 h + continuous over 80 days (total released: 0-45 %).	[206]

Note: Imp = impregnation; L = loading; R = release; A = activity; PEG = polyethylene glycol; n.a. = not available; * = estimated from the study data.

4.5.2. Encapsulation methods

Two techniques have been used for the encapsulation of BMP in silica: (i) the impregnation of already synthesized gels in a protein solution (*i.e.* impregnation method, **Erreur ! Source du renvoi introuvable.** (a)) and (ii) the direct incorporation of protein during the formation of the gel (*i.e. in situ* method, **Erreur ! Source du renvoi introuvable.** (b)). As the addition of the protein is dissociated from the matrix synthesis, the impregnation method allows the use of a wider range of synthesis and processing conditions, which gives more freedom for the design of the silica matrix [125,207,208]. In

this case, different parameters can be specifically optimized such as the pH, the solvent, the temperature, and the water-to-silica precursor ratio [136,209]. However, this technique requires a longer processing time.

On the opposite, in the case of the *in situ* method, the protein is directly added during the silica synthesis, reducing the processing time [125]. Yet, because of the presence of proteins in the sol, the synthesis and processing parameters must be carefully chosen in order to fit the restrictive conditions suitable for the protein, reducing the possibilities of tuning the silica matrix. In this case, extreme pH, high temperature, and non-aqueous solvent must be avoided. Another problem of this technique is the possibility of permanent entrapment of the proteins due to their high molecular weight [125,126,210].

4.5.3. Physicochemical properties of silica

To combine a high protein loading and a controlled protein release, the protein-matrix interactions must be carefully adjusted. Indeed, a high protein loading requires a high affinity of the protein for the delivery matrix. Yet, a high protein affinity for the matrix can lead to a high protein retention and even to a total entrapment of the protein in the pores. A compromise must therefore be found. To modulate the interaction between the protein and the matrix, two main characteristics of silica gels can be investigated: (i) the textural properties and (ii) the surface chemistry of the silica pores.

Textural properties

The textural properties play a key role in protein encapsulation and release. Indeed, pore size should be small enough to obtain a high specific surface area for protein adsorption but also large enough to allow proteins to diffuse from the protein solution towards the pores (in the case of the impregnation method) and from the pores to the release medium [125,128]. Additionally, physical and geometrical interactions between the pore walls and the proteins can also regulate the diffusion inside the pores and consequently the release kinetics. As already mentioned, the pore morphology can be controlled during the synthesis notably through the silica precursor, the pH, the quantity of water or the solvent [134,136,211].

The nature of the silicon alkoxide greatly impacts the kinetics of hydrolysis [134,136]. The increase in length of the alkyl chain leads to a decrease of hydrolysis rate due to a steric hindrance. The most used silicon precursors are tetraethyl orthosilicate (TEOS) and tetramethyl orthosilicate (TMOS). The influence of the silica precursor was shown in [206]. The authors showed that the adoption of different main silica precursors specifically affected the textural properties, especially the pore size, through the mechanism of silica formation (i.e. presence or absence of a nucleation process) and the reaction speed between the main silica precursor and the organosilane. The increase in specific surface area for the sample synthesized with TEOS compared to the one produced with TMOS. A faster and higher release of proteins was noticed for the silica synthesized with TEOS compared to the one produced with TMOS.

The addition of organosilane can also alter the textural properties of silica. This can be notably caused by the nature of the organic part (i.e. increase in reaction speed by acidic or basic groups) or its steric hindrance (i.e. a larger size of the functional group leads to a larger steric hindrance, which in turn results in larger spaces between nuclei and, hence, a higher surface area [206,212]).

Acids (*e.g.* HCl, HNO_3) and bases (*e.g.* NH_3) are used as catalysts to control the relative rates of hydrolysis and condensation [134,136]. Indeed, the reaction pattern of the hydrolysis step is influenced by the presence of H^+ or OH^- species [134,213,214]. Using an acidic catalyst, the hydrolysis step is slow, producing linear polymers with less siloxane cross-linkage bonds. By contrast, using a basic catalyst, the hydrolysis of the silica precursor is faster than in the acid medium, resulting in highly cross-linked polymers. In the case of structured silica, the pH affects the aggregation of the surfactant and in turn the pore structure.

The quantity of water can also regulate the silica structure. Indeed, an increase in the molar ratio H_2O:alkoxide accelerates the hydrolysis step, leading to a more cross-linked silica and a higher porous volume [136]. A solvent is usually used to homogenize and mix the aqueous and organic phases, which are generally non-miscible [136]. The addition of alcohol hinders the hydrolysis and condensation steps by promoting the reverse reactions (Equations 1 and 2).

Regarding silica structure, Musgo *et al*. showed that the pore size of the silica gel decreased by increasing the molar ratio ethanol:TEOS [215]. As described in Section 4.4., structuring agent like surfactants can be used to produce organized silica with narrow pore size distribution. The nature and the concentration of the surfactant greatly affect the pore size distribution and pore structure. Tilkin *et al*. [204] also showed that the addition sequence of the reagents had a great influence of the organization of the silica. The organization of silica and, in particular, its pore interconnectivity [204,216] greatly affect the encapsulation and release of proteins. In the case of non-interconnected pores, the proteins can only diffuse through the two extremities of a given pore, which can raise problems during the encapsulation and release processes. In the case of the encapsulation process, if several proteins adsorb on the same pore section, the pore is hindered, preventing further protein diffusion inside this pore. In the case of the release process, the diffusion of the proteins adsorbed at the center of a pore is blocked as long as there are remaining proteins in their path to the exit. On the opposite, in the case of interconnected pores, these blockages can be bypassed, which gives more opportunities for the proteins to diffuse inside the entire matrix or to escape the silica pores.

This phenomenon was observed by Tilkin *et al*. [204] when comparing the encapsulation and release of STI inside structured (SBA-15) and unstructured silica (Figure 6). The structured silica did not exhibit interconnection between the pores. If the two extremities of the pore are blocked, the entire pore is blocked, which reduces the surface area accessible for protein adsorption. On the opposite, in the case of unstructured silica, an interconnected network of pores was present. In this context, if blockage occurs, proteins can find an alternative route to diffuse throughout the entire matrix.

Finally, post-treatments such as calcination have a great impact on the pore size and surface area. Indeed, the increase in temperature will degrade the organic groups present at the surface of the pores, which increases the surface area of the calcined sample compared to the non-calcined one. Above a certain temperature, the silica sintering process occurs, which leads to a reduction of the specific surface area [119].

As a general trend, it was observed that a larger specific surface area led to a higher quantity of encapsulated proteins, as long as the pore size was high enough for the protein to get inside (for the impregnation method). Regarding the release, larger pore let to more rapid release of the protein. Pore interconnectivity is also a key factor. Yet, exceptions to these trends can be found, mainly due to the surface chemistry of the silica.

Surface chemistry

Specific functional groups, such as amine, carboxyl, alkane, and styrene can be incorporated at the surface of the pores to regulate the encapsulation and release processes [127,217–219]. Due to their composition, these groups bear a specific charge, have a specific hydrophobicity and take up a specific space. They can therefore specifically modulate the affinity of the protein for the surface through electrostatic forces, hydrogen bonding and van der Waals forces, and even covalent interactions [125,127,218,219]. This specific surface modification aims at creating an environment favorable to a large adsorption of the proteins and to a controlled release kinetics, while avoiding a permanent blockage of the release process (*i.e*. formation of covalent bonds) or an alteration of the protein activity (*i.e*. formation of strong interactions modifying the protein conformation). The composition of the silica surface must match the protein surface charge distribution, size, secondary and tertiary structures, hydrophobicity, and surface reactivity, and must therefore be specifically adapted to each protein.

The surface modification of silica pores can be realized via co-condensation, postsynthetic grafting, and the use of bridged organosilica precursors (Figure 7). The co-condensation technique is a one-pot synthesis in which silica precursors are mixed with organosilanes ($R–Si(OR')_3$) to include organic residues at the pore surface [220–225]. This technique allows an homogenous distribution of the functional groups without blocking of the pores in only one synthesis step [225,226]. Nevertheless, as already explained above, the addition of organosilanes can have an impact on the textural properties. In the case of structured silica, the degree of order of the materials decreases with the proportion of added organosilane. This can even lead to totally disorganized silica. Consequently, in order to keep an organized material, the proportion of organosilane should be limited to 40 mol.%. Furthermore, conventional surfactant removal treatments such as calcination should be replaced by softer treatments such as ethanol removal in order to avoid destructing the organic functions [226].

The post-synthetic grafting consists in the reaction of organosilanes, or less frequently chlorosilanes ($ClSiR_3$) or silazanes ($HN(SiR_3)_3$), with the free silanol groups at the surface of preformed silica.[225–230] This technique presents the advantage of maintaining the organization of the modified silica [226]. However, post-synthetic grafting leads to a decrease in the textural properties (*e.g.* pore diameter, specific surface area, and pore volume) of the porous silica, depending on the size of the grafted groups [225,226]. Moreover, blocking of the pores can appear if the organosilanes preferentially react at the pore openings during the initial stages of the synthetic process. From a practical point of view, this technique requires more steps and stricter conditions (*e.g.* anhydrous conditions) than the co-condensation technique [225].

Bridged organosilica precursors are organosilanes holding an organic functional linker between the silicon atoms, *i.e.* $(R'O)_3\ Si – R – Si(OR')_3$ [226,231,232]. These functions are included inside the walls after hydrolysis and condensation reactions [226,233]. A wide variety of bridges have been used from simple functions such as ethylene, or phenylene, and bis-phenylene, to complex bridges containing for example 1,4-diureylenebenzene, bipyridinium, or binaphthyl moieties [234–242]. These groups affect the rigidity of the walls and the general structural characteristics of the materials [242]. High loading and uniform distribution can be achieved, which allows an easy control of the physicochemical properties of the matrix while ensuring the structural stability of the matrix [241,243]. As for co-condensation, in the case of structured silica, soft surfactant removal techniques should be used due to the presence of organic functions inside the walls.

The accessibility of the functional group is also important. Indeed, the groups must be accessible to the protein to exert their influence. In this regard, the main silica precursor must be carefully selected. Indeed, Tilkin *et al*. [206] have shown that when using an organosilane containing methyl groups, TMOS simultaneously reacted with the organosilane, leading to a high accessibility of the functional groups. On the opposite, when using the same organosilane, TEOS reacted after the organosilane, forming particle composed of an organosilane core surrounded by a TEOS shell. The amine groups were thus covered and not as accessible as in the case of the samples synthesized with TMOS. Nevertheless, in the case of large organosilane/TEOS ratios, the authors detected the presence of accessible amine groups due to an insufficient amount of TEOS that could only partially cover these groups.

The conditions of encapsulation and release also have a signification impact, especially through the electrostatic interactions between the protein and the material. For silica, when the medium pH is lower than the point of zero charge (i.e. pH for which the material is not charged), the surface charges of the sample are positive, characteristic of the presence of groups like $–OH_2^+$ or $–NH_3^+$ [244,245]. By contrast, when the pH is higher than the point of zero charge, the surface charges of the sample are negative, characteristic of the presence of groups like $–O^-$ or $–NH^-$ groups. By playing on the medium pH, it is therefore possible to change the affinity of the protein for the material.

The post-treatments such as calcination is an important parameter to take into account. The calcination at high temperature [246] lead to the condensation of adjacent silanols to form siloxane bonds. The

proportion of silanols decreases with the calcination temperature. Siloxane groups are less acidic than silanol groups and tend to decrease to point of zero charge. In turns, this change in point of zero charge could lead to a change in surface charges, as explained above.

As a general trend, a high affinity of the protein for hydrophobic and negatively charged surface was observed, with a predominance of the effect of hydrophobicity. Indeed, samples modified with hydrophobic groups exhibited a high amount of encapsulated protein and a low release. Future development should focus on the control of the hydrophobicity of the pores, notably through the nature (e.g. phenyl, C18, mix with other groups), the concentration, and the position (i.e. pending in the pores vs included inside the walls) of the hydrophobic groups.

4.6. Deposition of silica at the surface of the implant

Two main strategies can be proposed for the deposition of silica at the surface of scaffolds: (i) the coating of the substrate with a silica film and (ii) the deposition of a dispersion of silica particles contained in another matrix. On the one hand, the first technique consists in the formation of a silica film at the surface of the 3D substrate. The process can be performed in two steps (*i.e.* film formation followed by immersion in a protein solution, Figure 8 (a)) like in the impregnation technique (Section 5.4.1.), or in one step (*i.e.* direct incorporation of the protein during the film deposition by addition of the protein in the sol, Figure 8 (b)) like in the *in situ* technique [103,195,196,201,247]. On the other hand, the second strategy is based on the dispersion of silica particles preliminary loaded with the protein, in a matrix by blending (**Erreur ! Source du renvoi introuvable.** 8 (c)) [248–251]. The matrix is then deposited at the surface of the 3D substrate. It is worthwhile to notice that particles have also been deposited on glass without a matrix [252]. Nevertheless, particles detach very easily from the support and particles uptake by the cells is already observed after 24 h. This rapid particle leaching from the implantation site is undesirable because leading to a release of the therapeutic protein outside of the targeted part of the body. Moreover, those particles represent a potential source of chronic inflammation with activation of macrophages.

From a practical point of view, the deposition of a silica film requires to synthesize and process the silica gel simultaneously. The synthesis process developed for bulk silica must therefore be adapted to fit the new requirements imposed by the processing step (*i.e.* reagent concentration, solvent) and the presence of the support (*i.e.* degradation during the film deposition or during the post-treatments). These modifications reduce the possibilities of tuning the silica matrix. These modifications influence the textural properties and surface composition of the silica, hence affecting the protein encapsulation and release. Moreover, more sophisticated apparatus like controlled atmosphere can be needed, notably in the case of structured mesoporous silica [253,254]. For these reasons, the dispersion of silica particles in a matrix followed by the coating of the composite on the substrate seems a very attractive technique.

In this context, the matrix should be selected in order to enhance the deposition of silica particles on the surface of the CaP scaffold. The matrix must also be biocompatible and should not impair the diffusion of the proteins released from silica. Polymers are a suitable choice for this application, as they have been shown to increase the solubility of drugs, improve their stability, and decrease the immune response of the body [255,256]. Moreover, the molecular architecture of polymers can be tailored to control their response to external conditions, their biodegradability, and their release rate [257,258]. The choice of polymers is further justified by the fact that bone is a composite composed of an inorganic (*i.e.* mainly HA) and an organic part (*i.e.* mainly collagen) [30,259]. Different matrices have been proposed for the dispersion of silica for drug-delivery applications, including poly(trimethylolpropane trimethacrylate) (polyTRIM), poly (methyl methacrylate) (PMMA), chitosan, polyvinyl alcohol (PVA), ethylcellulose (EC), polydimethylsiloxane (PDMS) [260–265]. Yet, BMP have not been used. A few examples are provided in Table 4.

Table 4 – Encapsulation of BMP-like proteins in silica gels.

Silica type	Polymer	Molec.	Encapsulation / release results	Ref.
SBA-3, SBA-15	PolyTRIM	Diclofenac sodium	L: 20 mg/100 mg; R: burst over 30 min then continuous over 25 h (total released: 40-85 %); A: n.a.	[261]
Propyl methacrylate modified silica	PMMA	Aspirin	L: 23 wt%; R: continuous over 90 days (total released: 20-70 %), rate increases with quantity of silica and decreases with quantity of modifier; A: n.a	[265]
Mesostructured silica nanoparticles	Chitosan	Bovine serum albumin	L: 78 % efficiency; R: continuous and total release in 24 h, rate increases with quantity of silica; A: no significant change in structure	[262]
Xerogel	Chitosan	Vancomycin	L: 23 wt%; R: continuous over 90 days (total released: 20-70 %), rate increases with quantity of silica and decreases with quantity of modifier; A: n.a	[264]
Amino-modified mesostructured silica nanoparticles	PVA	Bovine serum albumin	L: n.a.; R: continuous over 120 h (total released: 80 %), rate 3 times slower compared to polymer alone; A: n.a	[263]
MCM-41	EC + PDMS	Ciprofloxacin	L: 131 mg/g MCM-41; R: burst over 24 h then continuous over 30 days (total released: 19 % after 30 days), 4,4-fold reduction of burst compared to polymer alone, slower release compared to polymer alone; A: n.a	[260]
Xerogel	Chitosan	Vancomycin	L: 20 mg/g*; R: continuous over 30 days (total released: 40-100 %), decrease in rate with quantity of silica; A: n.a	[264]

Note: L = loading; R = release; A = activity; n.a. = not available; * = estimated from the study data.

Different techniques have been proposed for the deposition of films at the surface of substrates such as dip-coating [266,267], spin-coating [268,269], spray coating [270]. bar coating [271], and casting [272,273]. Among them, dip-coating and casting are of interest for the surface modification of tridimensional structures [274]. Dip-coating consists in the dipping of a substrate in a solution containing the matrix to be coated (*e.g.* a silica precursor and a catalyst in a solvent, polymer in a solvent) followed by its withdrawal at constant speed [266,267]. The thickness of the films is controlled by the viscosity of the solution and the withdrawal speed, *i.e.* increase of the film thickness with viscosity and withdrawal speed. From a practical point of view, dip coating is simple and presents a wide operating range (*e.g.* viscosity between 20 and 2000 mPa.s). Another advantage is the cheapness of the hardware. This technique is very promising as it allows the deposition of a uniform layer at the surface of biomedical devices. The main disadvantage of this method is the difficulty of modelling because of its time-dependent evaporation-induced concentration and viscosity gradients properties [136]. On the other hand, casting is a very simple method that is widely used for coating, especially using polymer. The technique is based on the casting of a liquid (*e.g.* solution, molten state) onto a substrate followed by solidification (*e.g.* drying, chemical reaction, gelation, cooling) [275–277]. This technique is usually used to produce thicker films. The film properties in this technique depend on the nature of the liquid

and the concentrations used. It presents some disadvantages like the lack of control over the film thickness.

5. Conclusions and perspectives

In this review, sol-gel silica has shown their great potential for surface modification of scaffolds for bone reconstruction. Indeed, sol-gel silica is an amorphous porous materials formed by an interconnected network of silicon atoms that can be synthesized at conditions not detrimental for biomolecules (mild temperature and pressure conditions). This material presents a large specific surface area, allowing many biomolecules to be adsorbed at the surface of the pores, more specifically, BMP, a biomolecule implied in the bone reconstruction.

Moreover, the morphology of the pores can be tuned depending on the synthesis conditions (*e.g.* pH, solvent, processing conditions) in order to regulate the release process via different interactions between the guest molecules and the host pore. Indeed, structured mesoporous silica can be tuned during sol-gel synthesis by using different surfactants. This process results in silica materials with very specific pore structure as hexagonal, cubic or lamellar. Their well-defined textural properties (*e.g.* ordered pores and homogenous pore size distribution) allow a large control of the diffusion of the biomolecule inside the pores and thus of the biomolecule release outside of the materials.

The surface of sol-gel silica materials can also be modified to add different charges or hydrophilic/hydrophobic groups in order to change its interactions with the protein (*i.e.* electrostatic forces, hydrogen bonding, van der Waals forces, covalent interactions). From a processing perspective, silica gels can be shaped in various configurations as films, monoliths or powders. Silica is also a biocompatible material with resorbable and non-toxic properties.

For the encapsulation of BMP in silica, two methods have been highlighted in the literature: (i) the impregnation on silica gels and (ii) the encapsulation during the material formation. The studies have shown that immobilization on silica and the subsequent release of BMP over time (up to weeks) is feasible. In the literature, the composition and the porosity of these materials are reported, but only a few studies link the textural properties and surface chemistry of silica with the release kinetics and remaining activity of the proteins.

To reach a high protein loading and to control its release, two properties of silica can be modulated: (i) the textural properties and (ii) the surface chemistry of the silica pores. The textural properties play a key role in protein encapsulation and release through its pore size, which needs to be large enough for protein to enter and diffuse inside and from the material but also small enough to reach high specific surface area. Surface chemistry of the silica can be modified with specific functional groups, such as amine, carboxyl, alkane, styrene, to interact with the protein via different mechanisms (as electrostatic forces, hydrogen bonding, …). The goal of this modification is to promote the adsorption of the proteins (in the case of the impregnation method) while slowing down the diffusion inside the pores, without totally blocking the release process (*i.e.* formation of covalent bonds) or altering the protein activity. The tuning of textural properties and surface chemistry must be specifically adapted to each protein to match its characteristics.

Finally, two main strategies were highlighted regarding the deposition of silica at the surface of scaffolds: (i) the coating of the substrate with a silica film and (ii) the deposition of a dispersion of silica particles contained in another matrix. The second option seems more feasible as the deposition of a silica film requires to synthesize and process the silica gel simultaneously. The synthesis process developed for bulk silica must therefore be adapted to fit the new requirements imposed by the processing step (*i.e.* reagent concentration, solvent) and the presence of the support (*i.e.* degradation during the film deposition or during the post-treatments). These modifications reduce the possibilities of tuning the silica matrix. Different methods exist for the deposition of films at the surface of substrates such as dip-

coating, spin-coating, spray coating, bar coating, and casting. Among them, dip-coating and casting are of interest for the surface modification of tridimensional structures.

When talking about the development of these new silica biomaterials, two essential steps should be considered: the sterilization and the *in vitro* evaluation. These steps are not always considered in literature but are very important. Indeed, the effect of the sterilization process on the physicochemical properties of the surface modification matrix (silica) as well as on the protein activity should be assessed. In case of significant degradation during the sterilization process, alternatives should be developed such as the use of sterilized environments. The *in vitro* evaluation of the implants is also critical for the determination of the biocompatibility of these new biomaterials and their approval by the authorities.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest.

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