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Sol-gel Encapsulation of Biomolecules and Cells for Medicinal Applications

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Abstract: The sol-gel process provides a robust and versatile technology for the immobilization of biologicals. A wide range of inorganic, composites and hybrid materials can be prepared to encapsulate molecular drugs, proteins, antibodies/antigens, enzymes, nucleic acids, prokaryotic and eukaryotic cells into bulk gels, particles and films. This review describes the applications of sol-gel encapsulation relevant to medicinal chemistry focusing on the recent development of biosensors as well as systems for production, screening and delivery of bioactive compounds and biomaterials.

Keywords: Bioreactors, Biosensors, Delivery, Drug, Encapsulation, Sol-gel, Tissue engineering.

1. INTRODUCTION

The immobilization of biological molecules within a three-dimensional matrix plays a major role in medicine. On the one hand, the encapsulation of active principles within carriers is a key step for the design of pharmaceutical products as it controls its spatial and kinetics delivery properties [1]. On the other hand, whereas most bioanalytical methods used for diagnostic purpose involve liquid phases techniques (especially chromatography) or reactive surfaces (such as traditional ELISA tests), the possibility to increase the loading and enhance the stability of sensing molecules upon storage and use via 3D immobilization is now well-recognized [2]. Considering living organism-based devices, 3D immobilization is beneficial for the enhanced and continuous production or testing of drugs within bioreactors [3,4] as well as for the development of tissue engineering biomaterials [5,6].

Whatever the target application, the chemistry of the encapsulation process must take into account the same requirements to insure the preservation of the biological activity during and after immobilization [7]. This implies to operate in mild temperature conditions, aqueous solution, limited ionic strength and to avoid any additives that may induce biomolecule denaturation or be toxic for the cells. In addition, the resulting encapsulation matrix should exhibit internal surface properties that are not detrimental to the molecule conformation or cell membrane organization. Finally, the host system must possess controlled porosity to guarantee the access of substrates and nutrients to the guest elements and the recovery of the products while avoiding the leaching of the active biologicals and, for certain *in vivo* applications, the internal diffusion of antibodies generated by the immune system.

Thus, the primary and limiting step of the encapsulation process is to achieve an aqueous polymerization or gelation reaction in mild conditions. This explains why biological macromolecules that form soft materials in nature have been, and remain, the favored source of reagents in bio-immobilization procedures [8]. This includes polysaccharides such as alginate, agarose or chitosan as well as proteins such as gelatin and collagen. In addition to their suitable chemistry, these molecules form hydrogels, *i.e*. highly hydrated networks that are well-adapted to favor the preservation of biological activities [9]. Biomacromolecules also have no eco-toxicity, many of them are non cytotoxic towards a wide range of cells and most of them are biocompatible. Yet, hydrogels suffer from relatively low mechanical properties, high swelling and poorly-controlled porosity. Indeed a number of techniques such as chemical, enzymatic or photo cross-linking, coacervation or composite approaches have been developed to overcome these issues [10].

Alternatively, the pioneer works of Braun and Carturan showed that the so-called sol-gel process was well suited for bioencapsulation purpose [11,12]. As will be described in section 2, this technology allows the formation of transparent gels in water and at room temperature. The progresses of this approach in a wide variety of fields, from biocatalysis to environmental remediation, have been timely summarized and discussed over the last decade [13-18]. In this review, emphasis has been placed on the application of the sol-gel bioencapsulation technology for therapy (section 3), diagnostics (section 4), biomolecular drug production (section 5) and tissue engineering (section 6). In each case, it was attempted to demonstrate the specificities of the sol-gel ap-

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proach on the behavior of the immobilized biologicals and, ultimately, on the properties of the resulting biofunctional material.

2. THE CHEMISTRY OF SOL-GEL BIOENCAPSULATION

2.1 A Brief Introduction to Inorganic Sol-Gel Reactions

The sol-gel reaction term can be used for almost any reaction involving a metal or metalloid element (M) that is able to form polymeric structures in solution through the formation of organic bridges [19,20]. Although non-hydrolytic processes are known, most sol-gel reactions occur in the presence of water and lead to the formation of M-O-M bonds. Basically, the precursor of the reaction should bear two or more hydroxyl (OH) groups or any function (Fn) that can be substituted by hydroxyl groups upon contact with water.

In the first case, the formation of metal-oxygen-metal bonds occurs readily through a condensation reaction that can in principle extend as long as M-OH groups are available

$$HO\text{-}M\text{-}OH + HO\text{-}M\text{-}OH = HO\text{-}M\text{-}O\text{-}M\text{-}OH + H_2O$$

$$HO\text{-}M\text{-}OH + HO\text{-}M\text{-}O\text{-}M\text{-}OH = HO\text{-}M\text{-}O\text{-}M\text{-}O\text{-}M\text{-}OH + H_2O \ldots$$

In the second case, a preliminary hydrolysis step is necessary

$$M\text{-}F_n + H_2O = M\text{-}OH + F_nH$$

In a majority of cases, F_n is an alkoxide group (OR) with R being an organic moiety and the reaction produces the ROH alcohol.

The progress of the condensation process is highly dependent on the reactivity of the M-O bond and therefore on the pH. Acidic conditions favor hydrolysis and growth of short linear chains while basic conditions lead to fast condensation of extended tridimensional polymers (*i.e.* a sol of particles) (Fig. **1**). Gelation occurs when the growing polymers connect to form a continuous percolated network. Here again pH plays an important role [21]. In basic conditions, percolation mainly originates from space filling by large particles and the resulting network has an open structure. In contrast, in acidic conditions, percolation occurs through aggregation of short chains, resulting in a dense network. To finish this brief introduction, it is important to point out that acidic/basic conditions refer to the pK_a of the M-OH bond so that, for silica with $pK_a(SiOH/SiO^-) \approx 6$, neutral pH is considered as a basic medium.

2.2. Sol-Gel Reactions in the Presence of Biologicals

As far as silicon alkoxides are considered, two different situations should be distinguished. The traditional tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS) are insoluble in water and release denaturating and/or cytotoxic alcohol (*i.e.* methanol and ethanol, respectively) during hydrolysis. In these conditions, the typical sol-gel reaction involves solublization of the alkoxide in the parent alcohol and addition of an aqueous solution at a specific pH to achieve hydrolysis/condensation reactions. Indeed, the presence of large amounts of alcohol makes this process unsuitable for the incorporation of biomolecules or cells. A first step towards a compatible process was achieved by demonstrating that the presence of the parent alcohol was not required to achieve alkoxide hydrolysis in a reasonable time if the hydrolysis reaction was performed under sonication [22]. In these conditions, the initial mixture consists of acidified water (pH 2) droplets in the alkoxide solution, so that a large interface exists between the two components, favoring hydrolysis. Nevertheless, the resulting sol is still highly acidic so that biological species cannot be added as such at this stage. Thus Dunn *et al.*, proposed to add buffer solutions containing the biomolecules to the acidic sol so that the gel is formed near neutral pH [23]. This protocol was a major breakthrough in this field and continues to be extensively used in the literature [13], with minor modifications. However, living cells are much sensitive than biomolecules to the presence of alcohol. Thus it was observed the membrane

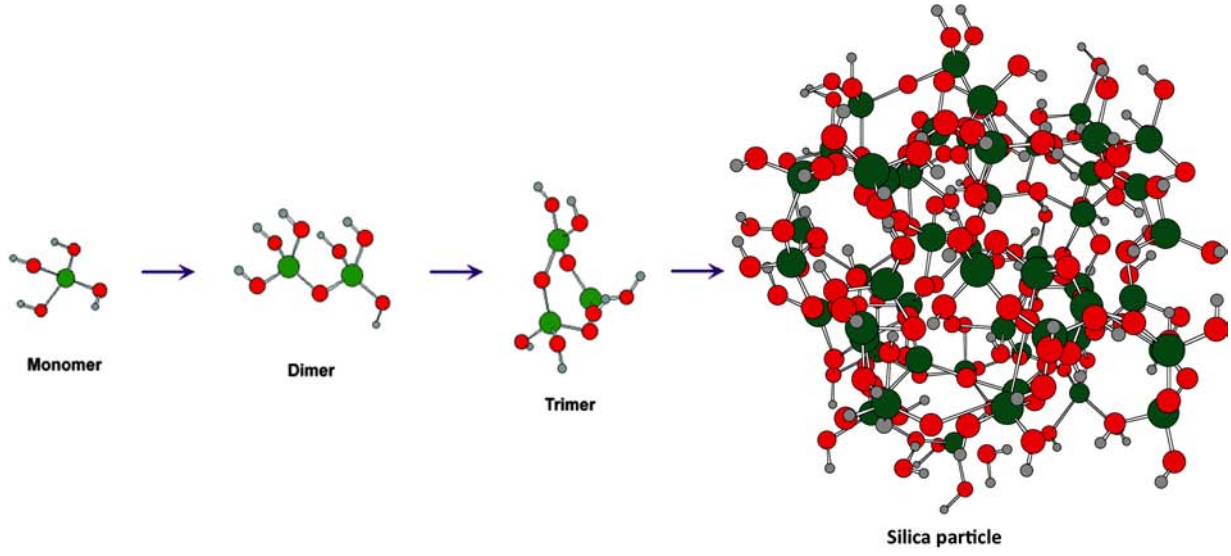

Fig. (1). The polymerization of silicic acid $Si(OH)_4$ leading to the formation of silica nanoparticles.

lysis of *Escherichia coli* bacteria encapsulated in silica gels prepared following this protocol [24]. This phenomenon was attributed to the alcohol released during the alkoxide hydrolysis. To avoid this problem, it is possible to perform the hydrolysis reaction under a nitrogen gas flow so that the released alcohol is continuously withdrawn from the sol as hydrolysis proceeds [25]. Another interest of this methodology is that the evaporation process is endothermic, avoiding any thermally-induced acceleration of sol-gel reaction and therefore stabilizing the sol. This approach was found suitable for the encapsulation of bacteria and micro-algae [26]. Finally, the alkoxide route exhibits the great advantage of using organically-modified silanes (ormosils), *i.e*. molecules of formula R_n'-$Si(OR)_{4-n}$, where R' is an organic group ranging from alkyl chains to amine- or thiol-bearing molecules. These groups can be used to tailor the properties of the silica network and of its surface, and ultimately the host-guest interactions [27].

The use of water-soluble alkoxide incorporating non cyto-toxic alcohols is more recent. Earlier reports from Gill and Ballesteros describe the preparation of polyglycerylsilane, a precursor that was found compatible with the encapsulation of many enzymes [28]. The same precursor was later used for cell encapsulation although the authors point out that it probably consists of a mixture of silanes rather than a well-defined molecular species [29]. Brennan *et al.*, extended this approach to several sugar-modified precursors (sorbitol, maltose, dextrose), all of which were found compatible with biomolecule encapsulation [30]. In parallel, tetrakis (2-hydroxyethyl) orthosilicate (THEOS) was successfully used for enzyme and later mammalian cells immobilization [31,32]. These precursors have a major practical interest as they can be directly dissolved in water at pH 7, in the presence of the cells, and form a gel within a few minutes. At this stage, their limitations are two-fold : (i) their hydrolysis/condensation reactions appear very sensitive to the presence of salts and (ii) although being non-cytotoxic, the alcohol/sugars may perturb specific cellular activity.

Very recently, a new kind of precursor, tetra(n-propylamino)-silane $Si(NH(C_3H_7))_4$, was evaluated for micro-algae immobilization [33]. Similarly to water soluble silicon alkoxide, these aminosilanes hydrolyze in water releasing n-propanolamine that exhibit limited cytotoxicity. However, the hydrolysis reaction is much slower than for alkoxide so that stable sols can be obtained on much longer periods before the gel is formed. First reports show limited cell viability but the approach was adapted for the immobilization of light harvesting complexes.

A second type of sol-gel precursors consists in metal salts. In the case of silica, the starting solution consists of aqueous alkaline silicates of general formula $M_xSi_yO_z$ (M = Na^+, K^+,..). These solutions contain large quantity of alkali stabilizing negatively-charged poly-silicic acids, *i.e.* oligomers of silicic acid $Si(OH)_4$ and are highly basic (pH 11-12) [34]. Neutralization of silicates solution by addition of a mineral base or concentrated buffer solutions allows the formation of a gel but in salinity conditions that exceeds ionic strength values compatible with many enzymes and, of course, most cells. In fact, mainly microalga cells that grow in saline environments can be safely encapsulated through this protocol [35]. To address this issue, a two-step method was described where the silicate solution was first put in contact with a H^+-cation exchange resin. The resin retains the alkali metals while releasing protons, leading to a slightly acidic silicate solution with low salinity. It becomes then possible to use buffer solutions to reach neutral pH and immobilize proteins or bacterial cells [36, 37]. Alternatively, the employment of citric acid instead of hydrochloric acid or cation-exchange resins in the condensation step was successfully employed for the immobilization of bacteria [38-39]. Citric acid is an intermediary of the tricarboxylic acid cycle and may also act as a compatible carbon source for bacteria or help bacteria under stress conditions to synthesize other protective solutes like glycerol. Indeed, cells grown in the presence of citric acid have been found to upregulate the enzyme glycerol-3-phosphatase leading to the synthesis of glycerol [40].

The third possibility relies on the formation of colloidal gels. This route is based on the use of sols of already-prepared well-defined colloidal particles whose gelation is induced by pH modification before addition or in the presence of the guest system. Such an approach has the advantage to permit cell immobilization in metal oxide gels whose molecular precursors, being alkoxide or salts, exhibit strong cytotoxicity, as demonstrated for aluminium-based matrices [41]. However, it also implies that the colloidal particles are not themselves cytotoxic. Moreover, it is not always possible to form colloidal gels in physico-chemical conditions compatible with cell survival [42]. Finally, it should be noticed that these gels tend to be easily redispersed as inter-particle interactions are weak.

Many alternative protocols have been derived from these three basic approaches. Mixtures of silicates and colloidal silica allowed the combination of the good stability of covalent network formation by the former with the low salinity associated with the later, leading to silica matrices compatible with bacteria immobilization [43]. Silicon alkoxides and silica particles mixtures were also evaluated for bacteria immobilization, although with limited success [44]. Mixtures of pre-hydrolyzed alkoxides and ceramic powders were also prepared and gelled by rapid casting on a cold plate (freeze-casting), leading to robust cellular hosts [26].

However, in many occasions, detrimental interactions between the precursor and the biological guest cannot be suppressed by simple adaptation of the sol-gel process. It then becomes necessary either to introduce additives in the sol-gel formulation or to pre-immobilize the target molecule or organism within a (bio)-organic scaffold before being put in contact with the inorganic precursors. Additives such as glycerol, poly-vinyl alcohol (PVA) or poly-ethylene glycol (PEG) can be used to control the polarity of the sol-gel environment, especially avoiding detrimental interactions between the biological elements and the silica surface [45]. The introduction of hydrophobic silanes, such as methyltrimethoxysilane (MTMS), also provides a convenient method to control the hydrophilic/hydrophobic balance of the host network, a crucial point when using interfacial enzymes such as lipases [46]. Another important issue is related to the osmolarity of the encapsulation solutions. Introduction of osmolytes such as sorbitol was found to stabilize

encapsulated enzymes as a result of their influence on the hydration state of the protein [47]. For bacteria, that are highly sensitive to osmotic pressure, the addition of glycine betaine, a well-known osmoprotector, provides a simple method to protect the cells against high salinity conditions [48].

The pre-immobilization of biologicals in a polymer network before adding sol-gel precursors avoids that the sol-gel reaction occurs at the contact with the cells but also insures that the cells are initially in a suitable environment for survival. This is particularly important for adhering cells that require a specific matrix (or at least specific adhesion sites). In this context, Carturan *et al.*, developed the Biosil process where Langerhans islets or hepatocytes initially trapped in alginate or collagen gels are placed under a continuous flow of air and silicon alkoxide vapors [14]. In these conditions, silica formation occurs only on the surface of the gel and the released alcohol is continuously withdrawn by the air-flow. Later on, solutions routes to achieve similar surface silicification were developed [49, 50]. To end with, an interesting approach was recently proposed by Perullini *et al.*, based on the entrapment of cell-containing alginate capsules (few mm in diameter) within bulk silica gels [51]. The capsules are then dissolved, creating macrocavities inside the inorganic network. This approach was found particularly useful to form non-silica gels, such as zirconia, whose precursors are highly toxic for the cells [52].

2.3. Gels, Films and Particles

As mentioned earlier, the progress of the sol-gel reaction ultimately leads to a three-dimensional porous inorganic network entrapping water (Fig. **2a**). Such hydrogels are particularly well-adapted for biologicals as they insure high hygrometry and diffusion paths for gas, nutrients and metabolites. However, from a practical point of view, the possibility to obtain thick (1-100 μm) to thin (10-1000 nm) films as well as to prepare micro- to nanosized particles are highly desirable.

In principle, all techniques that are currently used for preparing polymer hydrogel-based films such as drop casting, molding or spin/dip coating can be adapted to the sol-gel conditions. The preparation of thin films for enzymes and antibodies immobilization in silica has been reviewed recently, emphasizing the strategies that were developed to avoid fast drying and cracking, a major issue in inorganic films [53]. Extension to titania films was also recently described [54]. As far as cells are concerned, alkoxide and aqueous routes to thin films were also reported [55, 56] (Fig. **2b**).

The case of particles is different as it benefits or suffers from some specificity of the sol-gel reactions. Indeed, emulsion routes can be used to prepare beads, capsules and colloids of different sizes and are compatible with biomolecule encapsulation [57]. In contrast, room temperature aeorosol techniques that rely on the formation of droplets containing the network precursor and the biologicals and their gelation in a bath solution, a major technology for cell encapsulation, could not yet be adapted to sol-gel products due to the complexity of the hydrolysis/condensation reactions.

Coming to nanosized particles, many different ways exist to control the extent of the sol-gel reaction. In the case of silica, the most popular method was developed by Stöber, that takes advantage of the pH-dependent progress of the hydrolysis and condensation reactions [58] (Fig. **2c**). Silica formation proceeds from silicon alkoxide in an alcohol/water/ammonia media and the final particle size can be adjusted by the relative content of each component. This method can be applied to the encapsulation of molecules as long as they are not sensitive to high pH conditions and presence of alcohol. Moreover, the resulting loadings are quite low.

Currently, the most popular method for encapsulating biomolecules within inorganic objects relies on the preparation of mesoporous materials (MMs). MMs are obtained using a so-called templating approach where the sol-gel reaction is performed in the presence of a self-organized organic system (surfactants, polymers,…) [59]. The network forms around the organic template, which can be withdrawn afterwards, leaving open cavities whose size and organization, replicate the initial self-organized system. However most of these procedures are not compatible with the direct encapsulation of biomolecules. Thus, in a majority of cases, MMs are first prepared with a careful control of the pore dimension to fit with the size of the biomolecule to be immobilized. Then an impregnation step is performed where the guest system in solution is put in contact with the MMs as a powder or a film. Combining Stöber conditions or spray-drying techniques with templating approach allows the formation of mesoporous silica nanoparticles (MSNs) with pore size in the 20-200 nm range and various pore organization [60] (Fig. **2d**). Indeed these approaches are not strictly an encapsulation process as the host network is not formed in the presence of the guest molecule. Yet it has become an essential tool in the preparation of sol-gel based biofunctional materials, as shown in the next sections.

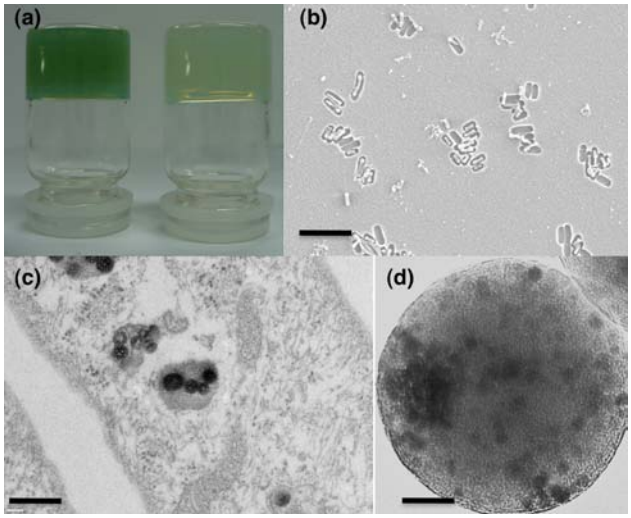

Fig. (2). Sol-gel materials for biology: (**a**) silica gels containing micro-algae; (**b**) bacteria immobilized in silica thin films (scale bar: 5 μm); (**c**) plain silica nanoparticles uptaken by fibroblast cells (scale bar: 500 nm); (**d**) mesoporous silica nanoparticles containing iron oxide magnetic colloids (scale bar : 50 nm).

2.4. From Immobilization to Release

At this stage, the different strategies available to encapsulate biomolecules and cells have been described. Then, depending on the targeted application, the bioactive component should either remain immobilized over a long-time period or be released in a controlled manner.

The first situation corresponds to biosensors and bioreactors. It requires the coupled optimization of the stability of the host network and of the preserved biological activity of the guest. As far as the materials are concerned, two main phenomena can occur, drying and dissolution/dispersion. The two processes are in fact related to the conditions of use and storage of the material, especially humidity level and temperature. For instance, silica dissolves much faster at 37°C than at room temperature [34]. The material stability also depends on their shape: drying rate increases with decreasing film thickness and dissolution rate increases with decreasing particle size or increasing porosity. In fact, the best method to improve the overall stability of a sol-gel material is to increase the extent of condensation within the silica network. This can be achieved by post-synthesis procedure such as ageing in controlled atmosphere, hydrothermal treatment or chemical reaction [19]. However most of these possibilities are not compatible with biomolecule-containing gels, not to mention living cells. One promising alternative is a so-called biomimetic approach that involves the use of natural catalysts of silica formation found in silicifying organisms such as diatoms and sponges [61]. These organisms build up robust silica-based materials that are stable in aqueous conditions [21]. Yet it is very likely that this stability is due to a combination of cellular components or products that are associated with the inorganic structure so that a lot of efforts are still needed to fully replicate these natural materials.

Preservation of biological activity on the long-term is another challenging task. In fact, enzymes and antibodies intrinsically exhibit short life-time in ambient conditions. Improvements in enzyme stability can prolong the lifetime of enzyme reactors, increase the potential for enzyme reuse, or maintain the good signal of biosensors [62]. The sol-gel network stabilizing properties derives from the high viscosity and the restricted mobility inside the matrix. It is generally accepted that the stabilization process is mediated through protein conformational flexibility limitation and the simultaneous generation of a protective micro environment [63-65]. Indeed, occurrence of appropriate environment around entrapped biomolecules is one of the important factors determining the functionality of entrapped proteins. Moreover, it was reported that the degree of protein hydration and local solvent composition can affect the structure and dynamics of proteins [66-68]. Moreover, the microenvironments of the sol-gel-derived biosensors in terms of elemental ratio, surface morphology, specific surface area, relative humidity and pore size were investigated to characterize the physicochemical properties of sol-gel immobilized biomolecules. [69-70]. A huge amount of literature reports that sol-gel encapsulation tends to prolong the time and temperature stability of biomolecules due to their confinement that limits conformational changes, provided that the gel composition and structure has been carefully adjusted [13, 71-73]. More details about the effect of encapsulation on the activity of the cells will be provided in following sections. From a materials point of view, two different situations are met depending whether cell division occurs or not within the silica gel. The first case is similar to traditional polymer-based encapsulation systems where proliferation can proceed as long as nutrients and oxygen are present, resulting in full colonization of the gel and/or leaching out of the matrix. The second case is quite specific to sol-gel materials [43]. As the inorganic pore size is smaller than the cell dimensions and the silica network is not biodegradable by most living organisms, cell division cannot occur so that biological means should be found to keep them alive as long as possible. Noticeably, this observation has long been a paradigm for silica-encapsulated bacteria until very recently. But recent reports showed that bacteria [56, 74] and hybridoma cells [32] division might occur provided that the inorganic network is flexible enough, *i.e*. when prepared from low concentration of silica precursors and in the presence of polyols.

The release of biomolecules from silica gels is a very different topic. Again, the principles are the same that apply to all production or delivery systems. The release rate can be tuned by adjusting the molecule-material interactions, creating suitable porosity inside the carrier and/or controlling the material degradation. However the sol-gel process is quite unique in the diversity of chemical functions and structural organizations that can be easily achieved using organosilanes and templating/self-assembly approaches. Many "smart" release systems involving the formation of stimuli-responsive bonds between the molecule and the host or the development of thermo- or biosensitive molecular valves blocking silica pores have been described [75]. In parallel, efforts have been made to understand and control the degradation of silica in biological environments, from biological fluids to intracellular environments [76, 77]. A number of examples will be provided in the next section to illustrate the capabilities of the sol-gel technology in this field. Nevertheless, it is worth closing this section by underlining again that most synthetic approaches to MMs and MSNs are not compatible with the presence of biomolecules in the reaction medium and must therefore be performed before the encapsulation step.

3. SOL-GEL ENCAPSULATION OF BIOMOLECULES FOR THERAPY

3.1. Silica-Based Materials to Deliver Growth Factors

Mesoporous Silica Nanoparticles (MSN) began to be studied as drug delivery systems in the early 00's when new processes permitted to control their morphology [78]. Success was first attained by Vallet-Regi and co-workers in 2001 when they managed to deliver Ibuprofen® from mesoporous nanoparticles [79]. Several modifications of MSNs have been performed over the following decade to render loaded particles stimuli responsive. For example, mesoporous channels could be capped by cadmium sulfides with the aim to trigger the drug release within the cells and not in the blood [80]. The recent developments in silica nanomaterials primarily aim to kill cancer cells within tumors [81]. Because of their textural properties (surface area, diameter and volume pore), MSNs allow high drug loading

capacities within their structure. MSNs are engulfed by multidrug resistant cancer cells in which high doses of drugs are released to kill them [82].

Several studies have been performed in order to deliver growth factors from silica xerogels or nanoparticles. The specifications for cytokine encapsulation are different from those of other molecules due to their action mechanism. These small proteins (less than 40 KDa) generally link to a specific cell surface receptor to trigger their biological effect. Unlike anti-cancer drugs, silica nanoparticles loaded with growth factors must not be internalized but must act as an external reservoir from which drug is released in the vicinity of targeted cells. Hence, the particle size should be big enough (more than 300 nm in diameter) or alternatively, the MSNs should be immobilized in a polymer bulk to prevent their internalization [81]. Protein loading within nanoparticles requires a pore diameter compatible with their encapsulation. Regular materials with mesopores below 3 nm in diameter prevent biomolecule encapsulation [83, 84]**.** To enlarge mesoporous, amphiphilic copolymers with greater molecular weight can be used. For instance, Pluronic F123 is used to produce MSNs with pore sizes between 5 and 9 nm. It has been shown that such large pores are suitable for growth factors loading as they allow the encapsulation of small proteins with a molecular weight up to 43 KDa [85]. Overall, pores larger than 4-5 nm are required for protein encapsulation. Other strategies rely on the introduction of expansion reagents or hydrothermal treatment [86, 87]. The second specification for an effective protein delivery is an adequate pore volume and suitable interactions with pore walls. Growth factors are macromolecules with tertiary structures. Their ability to bind their specific receptor relies on this structure. Hence, encapsulation within mesoporous hosts must not alter the 3D folding. By surface modifications of MSN with alkyl groups, Menaa and collaborators have shown that protein folding and biological activity are very sensitive to the hydrophilic/hydrophobic properties [88, 89]**.** Last, the loading within MSNs also depends on MSN/growth factors interactions, the pore volume and can be improved by several impregnations**.**

Growth factors are currently used in regenerative medicine to promote tissue repair. For instance, several studies have associated a drug delivery system with different bone substitutes to speed up wound healing. BMP-2 is currently associated with ceramics, bioactive phosphate or Bioglasses to promote tissue repair [90-92]**.** Another field of application for growth factors is cutaneous chronic wounds. These pathologies are characterized by chronic inflammation and a proteolytic environment [93]. It has been shown that cytokines such as PDGF or TGFβ1 promote wound healing in chronic wounds. However, all monotherapies based on injection of cytokines have failed due to the rapid diffusion and the short half-life of these molecules [94, 95]. In addition, a systemic application may result in high drug concentrations that would trigger harmful side effects. Encapsulation of growth factors within MSNs affords protection from the proteolytic environment and prevents the initial burst release. Unlike growth factors injection, drug release from large pore materials exhibited a zero order kinetic [92], an evidence of a controlled and delayed release. Bhattacharyya and coworkers have synthesized MSNs with pores expanded by a hydrothermal treatment (around 3.5 nm in diameter). Trypsin inhibitor (TI) was successfully loaded within MSN pores after impregnation [96]. TI was used as a growth factor model as its molecular weight is similar to that of common cytokines such as TGF-B, PDGF and BMPs. After loading with TI, MSNs were coated with PEG to prevent their opsonization by the immune system. MSNs provide a sustained release over 4 weeks without any burst release (zero order kinetic).

In tissue engineering, promotion of neovascularization is a critical step for the formation of a neotissue. Implants need to be rapidly vascularized to ensure cell colonization and remodeling. Basic Fibroblast Growth Factor 2 (bFGF) is one the major cytokines involved in blood vessel formation. Using a microemulsion technique, it is possible to generate bFGF loaded MSN with 7-10 nm mesopores [97]. As bFGF has an isoelectric point at 9.6, the biomolecule interaction with $Si\text{-}O^-$ allowed a high loading while preserving 3D integrity. MSNs could achieve a prolonged release of bFGF over at least 3 weeks with a small initial burst observed during the first 40 minutes. In addition, MSNs were not toxic for mammalian endothelial cells (HUVEC) and promoted their proliferation *in vitro*. Yet another strategy is based on the fabrication of core/shell magnetic mesoporous silica nanoparticles to associate a drug delivery system with magnetic properties. Beside the possibilities of tissue targeting due to magnetic properties**,** bFGF was released over 6 days without any toxicity on cells [98].

Silica-based mesoporous materials can be used in the form of films coating prosthesis or pre-formed implants. They are generated by dip or spin coating procedures. As an example, mesoporous silica film can be used as biomaterials for an application in the middle ear [99]. Several diseases such as chronic bacterial infections or tumor like aggregation of connective tissues lead to destruction of ear bones and hearing loss. The current treatment is based on a prosthesis implantation. However, some problems remain as a result of prosthesis displacement. It has been shown that the sustained delivery of BMP-2 from mesoporous film allowed for new bone formation and prosthesis fixation.

3.2. Sol-Gel in Gene Therapy

Gene therapy is considered as prospective treatment for the diseases caused by acquired and genetic disorders, such as AIDS and cancers. Nevertheless, the vulnerability of DNA and siRNA to hydrolysis, oxidation and so on in biological environment significantly impedes their efficacy. Efficient delivery vectors which offer substantial advantages including the prevention of degradation, possibility of active-targeting and controlled release are therefore in urgent demand. In this light, silica materials in forms of gels or particles obtained through sol-gel process have been proved the most promising inorganic materials in gene delivery systems.

The first study investigating sol-gel method for DNA encapsulation by Pierre *et al.* showing that small DNA molecules can be encapsulated within pure and hybrid polyvinyl alcohol-silica gels [100]. However, only limited amount of DNA could be re-extracted from these silica hosts, suggesting a molecular complexation between the silica and DNA after encapsulation. According to the results of NMR spec-

troscopy and nitrogen adsorption, the strong molecular bonding between the silica and DNA and small pore size of the gel were believed to be responsible for the limited release of DNA. To have a systematic study on the effect of presence and amount of DNA on gelation behavior of silica source, Kapusuz *et al.,* prepared silica-encapsulated DNA by mixing partially hydrolyzed TEOS solution and buffer solution containing model DNA [101]. It was found that sol-gel processing parameters such as DNA concentration and pH greatly influenced the gelation kinetics and microstructure of the silica. Besides, an effective encapsulation requires a size match between the DNA molecules and hosting pores. The conservation of the double-stranded form of DNA during sol-gel encapsulation was confirmed by accumulating DNA-intercalating molecules from aqueous solutions.

Fujiwara and coworkers encapsulated bovine serum albumin (BSA) and duplex DNA respectively into silica microcapsules directly by W/O/W emulsion [102]. Acidic salts added in water phase led to higher encapsulation capability, which was attributed to faster formations of silica shells. Despite the low encapsulation efficiency at around 30%, most of encapsulated BSA and DNA could not be released from the microcapsules without the destruction of microcapsule shell. Compounds larger than the pores of microcapsule shells could be encapsulated and therefore the as-synthesized hollow spheres have promising applications in enzyme immobilization, drug/gene delivery system and regenerative medicine.

Nonporous silica nanoparticles were used for the first time as inorganic nonviral gene delivery carriers by Kneuer *et al.,* in 2000 [103]. However, this study and most of the following ones focus on particle surface modification and adsorption of model plasmids by electrostatic interactions [104, 105]. Very recently, Paunescu *et al.,* proposed a method for encapsulating DNA into amorphous silica spheres to mimic the protection of nucleic acids within ancient fossils [106, 107]. DNA encapsulation was achieved by electrostatic adsorption and the following formation of silica shell. Positively charged N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TMAPS) was introduced before and after DNA adsorption to offset the repulsion of both negatively charged TEOS and DNA under reacting conditions. Within the silica nanoparticles, the nucleic acid molecules were perfectly sealed and therefore could survive high temperatures (as high as 140^{o}C) and aggressive radical oxygen species (ROS). Most amazingly, the encapsulated nucleic acids can be recovered intact by using fluoride-containing buffered oxide etch solutions and then analyzed by standard techniques such as gel electrophoresis and quantitative polymerase chain reaction (PCR) analysis. This interesting work may not only be applied in medical field such as gene therapy and gene storage, but also in products tagging and tracing in the market.

Mesoporous silica nanoparticles (MSNs) have extensive potential in gene delivery due to the large surface area, versatile functionality and excellent biocompatibility [108]. However, the loading of DNAs or RNAs inside the mesopores of MSNs seems a challenging mission and in most reports therapeutic genes were only adsorbed on the outer surface mainly due to poor availability of the mesopores [109-111]. Therefore, optimization of loading conditions and enlargement of mesopores were two most promising solutions to enhance the adsorption of genes inside the MSNs. Gu and co-workers investigated the most favorable conditions to pack the short salmon DNA and siRNA inside 3 nm mesopores of silica nanoparticles [112, 113]. The maximum loading of 12 wt% was achieved under chaotropic salt conditions, which theoretically can help to screen the electrostatic repulsions between the negatively charged DNA and MSNs. It was found that the increasing the salt concentrations and decreasing of pH value and temperature can promote the adsorption into the mesopores. Interesting, under the optimal conditions for DNA, almost no siRNA was absorbed inside the mesopores, suggesting different physiochemical characteristics between DNA and siRNA. Further investigation showed dehydrating conditions could lead to siRNA loading as high as 27.5 wt%. After adsorption, the mesopores were then caped by 25 kDa PEI, which showed efficient protection against enzymatic degradation. Very recently, the system was improved by grafting the system with a fusogenic type peptide, KALA, which is endowed with excellent endosomal escape capability [114] (Fig. **3**). In this case, PEI worked both as the cap and co-reactant for KALA. The as-synthesized delivery systems could not only protect siRNA from degradation, but also release it readily into the cytoplasm. The successful down-grading of enhanced green fluorescent protein (EGFP) and vascular endothelial growth factor (VEGF) were observed *in vitro* and the system loaded with VEGF-siRNA could effectively inhibit the growth of tumor over a month.

Kim *et al.,* reported high loading capacity of plasmid DNA could be achieved using monodispersed MSNs with a mean mesopore diameter of 23 nm (MSN-23) [115]. These MSNs enabled efficient gene delivery of two plasmids (pLuc and pGFP) *in vitro*. The efficient cellular delivery of siRNA targeting GFP and VGEF using MSN-23 as transporting vehicle was also reported [116]. It was found that the loading capacity of siRNA was twice higher than that of MSN with 2 nm pore size despite its surface area was only 1/6 of the latter. Incubated with RNase for 2h at 37 ^{o}C, the siRNA was intact after heparin-mediated detachment from the mesopores, implying successful packing of siRNA. The mean GFP expression level *in vitro* decreased to 12% upon treatment with siRNA-MSN-23 complex *in vitro* and decreased to 42% *in vivo* compared with that treated with PBS. The efficiency of inducing silence of VEGF was examined *in vitro* and was revealed by reverse transcription polymerase chain reaction (RT-PCR). Finally, the knockdown of VEGF *in vivo* was investigated by monitoring the tumor weights and VEGF mRNA levels over a period of 20 days. Compared with the tumor treated with PBS, the tumor weight was 80% less and the VEGF mRNA level was 85% lower.

Gao *et al.,* also successfully developed a large-pore silica nanoparticles based gene delivery system by applying the combination of low temperature (10 ^{o}C) and dual surfactant templating [117]. Low synthetic temperature was found indispensible for the creation of large internal cavities and high post-treatment temperature ensured the wide entrance of the mesostructures. To confirm the plasmid could be incorporated inside the cavities, methylation treatment was carried

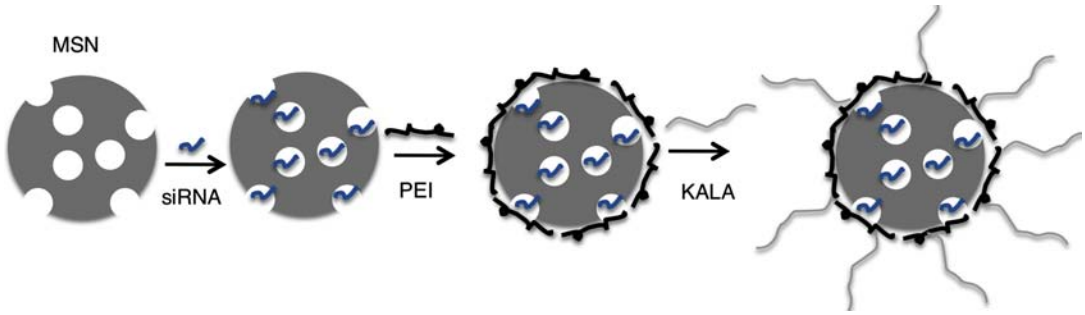

Fig. (3). Mesoporous particles for siRNA delivery. The siRNA is first immobilized within the pores of the particles. Coating by polyethyleneimine (PEI) allows the blocking of the pores and provides a suitable surface for grafting of the KALA peptide. Adapted from [114].

out to block the external silanol groups so that the amino group functionalization could only happen inside the mesopores. Interestingly, 76% of the initial loading was obtained using the as-synthesized MSN, suggesting most of the plasmid was absorbed inside of the pores. Further on, the as-synthesized large pore silica nanoparticles were grafted with poly-L-lysine (PLL) and aminopropyl-triethoxysilane (APTES) respectively to deliver oligo DNA-Cy3 (a model for siRNA) to Hela cells [118]. According to the thermogravimetric analysis, the amount of APTES grafted was almost twice that of PLL, yet the two systems show similar adsorption capacity of oligo DNA, as high as 57 μg/mg. The specific binding to A-T (adenine-thymine) sequences of DNA and 3-D reactive structure were considered to be responsible for higher affinity of oligo DNA to PLL than APTES, whose binding to DNA relies on electrostatic interactions only. Moreover, PLL-containing MSN showed a higher reduction of cellular viability of oncogenes in cancer cells at 30%, due to the better cell internalization and endosomonal escape.

To end with, it is worth mentioning the first and the only example of silica nanotubes explored for gene encapsulation [119]. Silica nanotubes with an outer diameter of 200 nm and length of 2 μm were prepared using an anodic aluminum oxide membrane as a template. The nanotubes, possessing a hollow structure and inner modification with aminopropyl-trimethoxysilane, were loaded with GFP plasmids and successful GFP expression was achieved in monkey kidney cells. Although the transfection efficiency was lower than that of calcium phosphate, better performance could be expected by adjusting the size and improving modification of the surface.

3.3. Sol-Gel Materials for Antibiotics Release

Post-operative infections following orthopaedic surgery still affect 3% of patients. Osteomyelitis (bone infection) is characterized by an inflammatory destruction and a necrosis that evolves towards a chronic state. An implant infection requires implant removal that impacts on both the patients' quality of life and on healthcare costs. The most common pathogen responsible for osteomyelitis is *Staphylococcus aureus* because it can form biofilms inside infected bone. Bacteria within a biofilm exhibit an altered phenotype that drastically modifies their antibiotic susceptibility. To achieve the therapeutic effect after systemic injection, high antibiotic doses are required and these are associated with toxic effects. Hence, local treatments based on the combination of a biomaterial with a drug delivery system have been studied. The aim is to deliver high doses of antibiotics to specific bone to favour its tissue repair. Poly(methyl metacrylate) (PMMA) bone cements are commercially used to fix prosthesis to living tissues [120]. These materials can be loaded with antibiotics by impregnation or mechanical mixing. Unfortunately, cements deliver drugs over a few days and only 5 % of the initial dose is released. In addition, PMMA is not biodegradable which necessitates a second surgery to remove the material when the infection is healed [121]. Alternatives have been found with the development of bioactive cements, calcium phosphate and hydroxyapatites. Nevertheless, these materials have some limitations such as a "burst" release due to their drug loading by impregnation. The ideal drug delivery system must be inert, stable, biodegradable, biocompatible and simple to administer. Moreover, this system has to have high loading capacities and must deliver antibiotics in a controlled and sustained manner. Controlled release reduces dosing frequency and eliminates some of the toxic effects associated with the systemic administration of free drugs.

Sol-gel technology permits production of xerogels (*i.e.* dried gels) at room temperature in aqueous media. Therefore, this process is compatible with biomolecule encapsulation such as antibiotics [122,123]. In addition, silica xerogels are stable, biocompatible, simple to synthesize and have a controlled degradability. For these reasons, silica xerogels appear promising for an application in the biomedical field as a drug delivery system. For an application in bone healing, drug release systems have been studied with two main antibiotics: gentamicin and vancomycin. Vancomycin has a high efficacy against gram-positive bacteria such as *S. aureus*. Gentamicin belongs to the aminoglycosides class, antibiotics family with a broad spectrum of activity on bacteria.

Mesoporous xerogels can be produced with a highly ordered porous silica structure. Because of their pore structure, these materials exhibit high loading capacities and a homogenous antibiotic distribution. Xerogels possessing hexagonal pores of 6 nm in diameter can be produced and used in the form of powder or disk [124]**.** Then, materials are loaded by impregnation in a solution of gentamycin. Despite good loading capacity (20% of the initial dose), these xerogels do not permit a controlled release from the material because the entire dose is released within 5-10 hours and a huge initial burst is observed. The release kinetic is antibiotic-dependent and is related to the strength of interaction between antibiotics and matrices. Nevertheless, drugs are not

retained for more than 24 hours within the matrix [125]. To improve the drug retention within materials, xerogels with pores smaller than 2.4 nm have been studied [126]. Moreover, matrix functionalization by incorporation of aluminium in the silica framework has been carried out to strengthen the interactions between matrix and antibiotics. Loaded with amikacin, an antibiotic belonging to the aminoglycosides, mesoporous xerogels did not allow for the controlled diffusion of the drug regardless of the aluminium content. The dose was released within 3-4 hours in the best conditions.

Another technique has been developed to delay drug release. This process relies on the dissolution of the antimicrobial agent in the structure-directing agent (block co-polymer P123). The antimicrobial peptide LL-37 is used in a modified mesoporous silica film monolith [127]**.** The incorporation of SH groups in the pore wall permits a slow release reaching a maximum after 200 hours with no initial burst being observed. The antimicrobial peptide is released in its active form as a bactericidal effect was observed on *S. aureus* and *E. coli*.

To prevent bone infection, antibiotics have to be released over a long period around the implant, *i.e* several weeks. Microporous xerogels (pore radius < 1 nm) allow a tunable drug release by variation of synthesis parameters [122,123]**.** Silica xerogels are prepared by mixing antibiotics with hydrolysed alkoxysilane in acidic conditions, followed by gelation, aging and drying. Vancomycin can be encapsulated with a high dose without precipitating and modifying the xerogel structure. The effect of the water/alkoxysilane ratio on antibiotic delivery has been analysed. When this ratio increases, drug release is faster due to an increase of the pore volume. Water enters the xerogel matrix, dissolves antibiotics and silica walls, allowing for drug diffusion. From a ratio of 6, the drug release kinetic exhibits a biphasic profile without initial burst. A faster "first order kinetic" is observed followed by a near-zero-kinetic. After 42 days, 90% of the dose is released irrespective of the initial loading. The effect of the drug loading is the delay for the second phase occurrence. Vancomycin released from xerogels maintains its activity as a strong inhibitory effect is observed on bacterial growth of *S. aureus*. Drug release kinetic can be modulated by modification of the degradation rate. For example, when a more hydrophilic amino-polysiloxane matrix is synthesized, this matrix behaves like a hydrogel. First, the matrix swells and then degrades to release its vancomycin content over one week [128]. The kinetic profile is characterized by a lag phase followed by a sustained release. Physical parameters also have an effect on the xerogel microstructure and on their drug release behaviour [129]. For example, a larger drying surface and a higher drying temperature cause considerably less cross-linking of the sol, resulting in a weaker structure. As a consequence, pores collapse and this leads to a denser structure. In these conditions, drug release is more delayed and controlled. Lastly, drug loading also influences release kinetic, *i.e* a high loading speeds up the release [128,129].

In vivo studies have been carried out on bioactive sol-gel glasses loaded with gentamicin. For instance, these material implanted in a bone defect in rabbit are able to deliver antibiotic doses higher than the MIC (Minimum Inhibitory Concentration) for 12 weeks around the implant [130]. Interestingly, gentamicin is only detected in kidney and lung for 2 weeks post-surgery, evidencing the localization of the drug delivery.

Sol-gel technology can be performed to produce silica nanoparticles that are able to deliver antibiotics after systemic injection. MSNs are of interest to deliver antibiotics in the blood or in the organs as they are inert, stable, biocompatible, and have a large surface area and a high pore volume. Unlike the encapsulation of proteins, regular MSNs with small pores can be used because antibiotics are not molecules with a high molecular weight. Moreover, MSNs can be functionalized to target different organs and deliver their drug content specifically. Unfortunately, antibiotics are not retained over a long time in mesopores. Lai and collaborators have overcome this leaking phenomenon by capping mesopores with cadmium sulfide crystals [80]. Nanocrystals and MSNs are linked *via* disulfide bonds that allow the stimuli-responsive release of vancomycin. Incubated in aqueous buffer, no vancomycin is released until the addition of reducing agent (DTT or mercaptoethanol). Then, the antibiotic content is rapidly released within 24 hours, evidencing the poor interaction of the drug with pores. The major improvement in stimuli-responsive MSNs is obtained with capped materials showing zero release in blood and able to deliver their cargo only in presence of bacteria [131]. Nanoparticles surface is first functionalised by carboxylate groups. Then, the particles are loaded with vancomycin and capped with a cationic polymer, poly-lysine. MSN capping greatly increases toxicity against gram-negative bacteria. The effect is attributed to the displacement of poly-lysine, which interacts with the bacteria membrane and allows for the release of high vancomycin doses near bacteria.

Mesoporous nanoparticles can also be used to improve drug delivery from PMMA-based cements [132]. MSNs loaded with gentamicin are mixed with PMMA to form a nanocomposite. Below 6 wt% in the formulation, MSNs are isolated and do not allow drug release. When the MSN content reaches 8 % in the formulation, gentamycin release is effective over 80 days. A linear release is observed over the first 20 days followed by a slower phase to reach a release of 70% of the initial dose. The MSNs incorporated in the cement form a nano-network path for gentamycin diffusion.

4. SOL-GEL ENCAPSULATION OF BIOMOLECULES FOR DIAGNOSTICS

4.1. Immunoassays in Sol-Gel Materials

As underlined earlier, the main advantage of the sol-gel bioencapsulation process is that it provides a 3D environment that stabilizes encapsulated biomolecules via steric hindrance of conformational changes. This offers the possibility to design biosensors with prolonged life-time and, in some cases, higher sensitivity [133].

In the case of immunoassays, ELISA methods based on antibodies adsorption onto well plates associated with optical detection are currently widely-used. Hence first studies of sol-gel encapsulation of antibodies mainly aimed at demonstrating not only the feasibility but also the advantages of this technology. In order to avoid limitations related to the diffusion of large antigenic species within the silica network,

these preliminary experiments were performed using available or specifically-prepared haptens [134,135]. Overall, a decrease in antibody-hapten affinities was observed but a significant increase in stability under storage and possible sensor regeneration were achieved. In parallel, it was shown that immunoglobulin IgG could diffuse into silica gels and react with antigenic sites present at the surface of encapsulated dead parasite cells [136]. This possibility was applied to the development of serodiagnosis assays for visceral leishmania and Trypanosoma cruzi [137].

Further progresses were made by using electrochemistry-based detection methods. The concept is to couple the target analyte with a redox enzyme (or in case of sandwich assays, to prepare an anti-target antibody coupled with this enzyme) and to detect its activity via an amperometric method in the presence of a specific substrate. This indeed requires that the sol-gel medium acts as an electrode. Since silica is not conductive, addition of graphite powder to the sol-gel formulation [138] or sol-gel deposition on a glassy carbon electrode were successfully employed in electrode performance (GCE) [139].

However, for practical reasons, the development of reagent-less sensors is highly desirable. This requires the optimization of the direct electron transfer between the redox enzyme and the electrodes. The works of Ju *et al.* nicely illustrates the different strategies that can be applied for this purpose. Initially, human serum chorionic gonadotrophin (hCG) was immobilized in a sol-gel titania film deposited on a GCE. Upon contact with an horseradishperoxidase (HRP)-labeled anti-hCG antibody, it was possible to evidence the formation of the immune complex via a direct transfer between the HRP and the electrode using cyclic voltammetry (Fig. **4a**) [140]. hCG detection was then performed via competition assays with the labeled antibody, reaching a detection limit of 1.4 mIU.mL^{-1}. In a step further, a sol-gel titania film was formed on GCE incorporating the HRP-labeled anti-hCG antibody together with gold nanoparticles (Fig. **4b**). Metallic particles were good substrates for antibody adsorption, therefore favoring the electron transfer from HRP to the electrode materials. In these conditions, upon addition of hCG, an immuno-complex was formed that hindered the electron transfer, as monitored by cyclic voltammetry measurements. In these conditions, the detection limit decreases down to 0.3 mIU.mL^{-1} [141]. As an alternative, a new silica-based formulation was developed involving TEOS and APTES to obtain a nanoporous material with good surface affinity for HRP, allowing to reach a similar detection limit [142]. Then, a combination of the two processes, *i.e.* incorporation of HRP-labeled antibodies in a TEOS/APTES/gold formulation was successfully applied to the simultaneous detection of carcinoma and carcinoembryonic antigens as tumor markers [143]. In this case, the sol-gel film was deposited on a graphite multiple electrode array and up to 4 markers could be monitored, with detection limits of ca. 0.5 U.mL^{-1}. Ultimately, the authors used an electric-field driven incubation strategy to improve the detection delay [144]. This strategy is based on the application of a potential on the electrode, creating an electric field that favor the diffusion of charged antibodies from the solution towards the sensing device. Through this approach, it was possible to lower the analytical time from 40 min to 2 min.

Another important application of sol-gel encapsulation of antibodies is immuno-purification allowing the separation of a target molecule from a complex medium, such as biological fluids or contaminated waters, through sorption and then its recovery for analysis by chromatography or ELISA tests [145,146]. In contrast to immunosensors, the silica material only acts as a solid support for the antibodies so that sol-gel formulation is optimized in terms of porosity and stability.

This was studied in details in a series of works by Altstein *et al.*, dedicated to the immunopurification of steroid hormones, such as levonorgestrel (LNG) and medroxyprogesterone acetate (MPA), as well as non-steroid anti-inflammatory drugs such as indomethacin (IMT). Typically, it consists of a TMOS aqueous solution pre-hydrolyzed in HCl, to which PEG is advantageously added to increase internal porosity while limiting non-specific adsorption [147]. After addition of antibodies in a buffer solution, gels are left to age over 24 h and are then crushed and packed in chromatography columns. Through this method, it was possible to obtain effective binding of LNG and to obtain a recovery rate of 75-100 % after elution in ethanol. However, it was

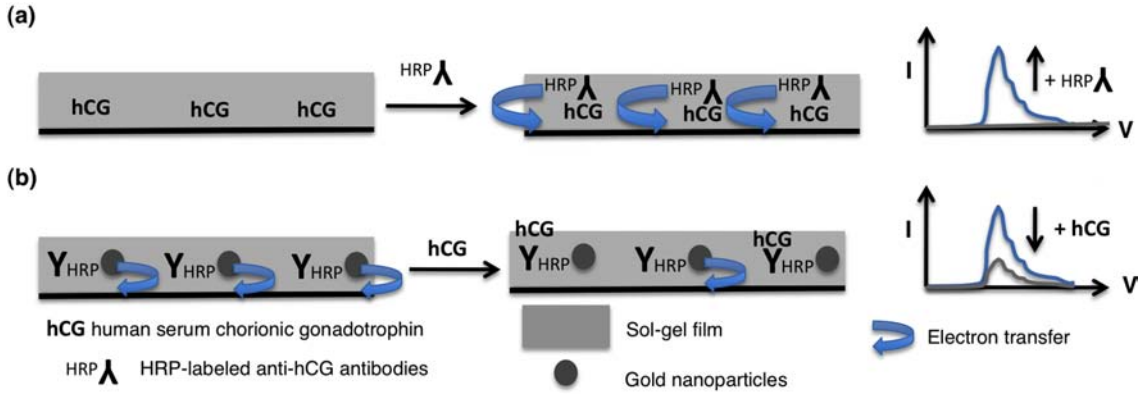

Fig. (4). Sol-gel based electrochemical immunosensors: (**a**) human serum chorionic gonadotrophin (hCG) is immobilized within a sol-gel film. The binding of the Horseradish peroxidase (HRP)-labeled anti-hCG antibodies is detected by electron transfer between HRP and the electrode, as monitored by an increase in the current peak intensity of voltamograms. (**b**) HRP-labeled anti-hCG antibodies are immobilized in a sol-gel film containing conductive gold nanoparticles. The binding of hCG hinders the electron transfer between HRP and the electrode, as monitored by a decrease in the current peak intensity of voltamograms. Adapted from [140,141].

found that the immobilized antibodies showed a significant cross-reactivity with parent steroid hormones, ethynylestradiol and nortestosterone, that was not identified in the ELISA test performed on the free antibodies [148]. The method was afterwards applied to the detection of IMT. Thanks to high drug binding (100-250 ng), low non-specific binding (< 5%) and efficient recovery, it was possible to perform the quantification of IMT in spiked human serum samples after immuno-sorption on sol-gel columns and extraction [149]. Interestingly, the eluted samples could be directly analyzed by LC-MS/MS. Similar results were more recently reported for MPA recovery and detection [150].

To end this section, it is worth mentioning recent works involving pre-formed porous materials. Indeed, the usual pore size range of mesoporous silica nanoparticles (2-20 nm) make them suitable for the immobilization of antibodies. This property has been used for the design of controlled release systems for cancer immunotherapy [151]. Surface grafting of antibodies on MSN was used for cell targeting purpose [152], as well as to design immune recognition-based smart delivery systems [153]. In the bioanalytical field, antibody-conjugated MSNs containing silver nanoparticles were used to improve the sensitivity of electrochemical immunosensors [154]. However, to the best of our knowledge, the achievement of immonoassays inside templated mesoporous materials has never been reported so far very probably due to steric issues. However, there is another kind of sol-gel materials, termed aerosols, which can exhibit mesopores. These materials are prepared by controlling the solvent withdrawal from the gel phase, mainly via supercritical drying approach, in order to avoid pore shrinkage [155]. In a recent report, such aerogels incorporating APTES were prepared, crushed and deposited as multiple array-dots on a glass slide [156]. Anti-human IL-6 could be immobilized inside the pores of the aerogels and the binding of human IL6 could be monitored via a sandwich assay.

4.2. Sol-Gel Encapsulation of Nucleic Acid Aptamers

As for antibodies, nucleic acid-based diagnostic tools involving sol-gel encapsulation have been developed taking into account that a very efficient technology already exists, *i.e*. DNA chips. However, whereas the 3D immobilization of antibodies had an evident advantage over traditional ELISA plates because the later are mainly based on protein adsorption, the DNA technology involves covalent grafting on 2D plates, combining suitable immobilization and rapid hybridization. Hence whereas a few reports are dedicated to the possible improvement of chips using sol-gel technology coating combined with surface grafting [157,158], most efforts have been afterwards devoted to identify original detection methodologies that cannot be achieved in 2D configurations [159].

The encapsulation of single strand ss-DNA within silica was initially described for micro-array development [160] as well as for separation purposes [161]. With undergoing developments of the aptamer technology, Kim *et al.* could design original and efficient bis-phenol A (BPA) biosensors. The initial sol-gel formulation based on TMOS, MTMS and PEG originated from a preliminary screening approach applied to protein-based chips [162]. However it was found that the addition of glycidopropyltrimethoxysilane (GPTMS) improved the stability and detection performances of silica gels containing ss-DNA aptamers targeting TATA sequences [163]. In a next step, BPA-sensitive aptamers could be obtained by the SELEX process and immobilized in sol-gel spots [164]. Based on sandwich assay involving BPA binding to the silica, followed by addition of cyanine 3 (cy3)-labeled BPA aptamer. Fluorescent detection was effective in the nM range. In parallel, although it does not involve biomolecule encapsulation, it is interesting to note that, as an alternative, BPA was immobilized in sol-gel plots and detected using fluorescence by simple addition of the cy3-labeled aptamer [165]. In this situation, the silica materials were deposited onto porous silicon substrates to favor their adhesion. Interestingly the apatmer recovery could be achieved under mild heating conditions. This suggests that these novel arrays can be particularly useful for aptamer screening.

Another interesting approach was developed by Brennan *et al.*, using fluorescent signaling DNA aptamers [159]. The initial tripartite construct consists of an ATP-targeting aptamer sequence associated with a fluorescently-labeled complementary ss-DNA and a small complementary oligonucleotide sequence tagged with a quencher. At the beginning of the experiment, the proximity of the ss-DNA and oligonucleotide associated with the aptamer leads to fluorescence quenching. However, upon ATP binding, the structure switch of the aptamer releases the oligonucleotide and therefore allows the detection of the fluorescent ss-DNA signal. A bipartite strategy involving direct grafting of the fluorescent moiety on the aptamer was also evaluated. In both situations, the silica-encapsulated constructs showed preserve ability to undergo structure switching upon ATP addition [166]. Recently a similar approach was successfully applied to RNA aptamers [167]. This result constitutes a major advance since the use of RNAs in biotechnology is limited by their strong sensitivity to nucleases. Here the nuclease attack was avoided by the encapsulation process. In this condition, it becomes possible to perform solid phase enzymatic assays using aptamers that are specific to the substrate of the enzymatic reactions. In the reported work, the adenosine deaminase enzyme and adenosine-sensitive aptamer can be either entrapped in two silica layers or co-immobilized in the same silica matrix [168]. Upon addition of adenosine, an initial high fluorescence signal is detected that decreases with time as adenosine is consumed by the enzyme reaction. However it remains constant if an enzymatic inhibitor is present. Coupled to micro-arrays techniques, this should allow to perform fast inhibitor screening tests.

Preformed porous silica materials were also used for DNA aptamer-based detection devices. Macroporous silica films were obtained from sodium silicate and PEG [169]. Structure-switching fluorescent aptamer releasing a quencher-labeled nucleotide (corresponding to bipartite systems described above) was then grafted on the surface *via* a strepatividin-biotin strategy. The amount of grafted aptamer was increased by a factor of *ca*. 5 compared to 2D glass substrates, resulting in an enhanced sensitivity. An alternative approach for the detection of small analytes was recently described using long-period grating sensors [170]. Such sensors are optical devices involving the monitoring of the

change in evanescent waves transmission at the surface of optical fibers upon modification of the refractive index of the medium. In this work, a porous silica coating containing in situ-grown gold nanoparticles was deposited on the fiber surface. It was shown that gold nanoparticles improve the optical sensitivity of the device as it enhances the refractive index modification upon nucleotide release. Further enhancement was obtained by using a quencher-labeled oligonucleotide. To end, it is worth mentioning that aptamer-grafted monolithic capillary columns were also recently described and applied to thrombin recovery from spiked human serum [171]. The tandem or on-line coupling of affinity recognition technologies with the high-resolving power of separation techniques is recognized as a powerful analytical tool for the enrichment and quantification of ultra-low abundance analytes in complex matrices [172-174].

A final comment should be made about mesoporous silica nanoparticles. As underlined earlier (section 3.2), nucleic acids have been introduced within MSN porosity for the purpose of gene delivery. In addition, as already described for antibodies (section 4.1.), surface-grafting of silica particles with aptamers can been used for cell targeting [175] and substrate-specific drug delivery [176]. Coming back to the bioanalytical field, a very recent report described an interesting option for detecting thrombin in human plasma and serum [177]. The strategy is based on the capping of the pores of a particle incorporating fluorescent dye with a thrombin-specific aptamer. In the presence of the target, the structure switch opens the DNA cap and releases the dye that can be measured in solution.

4.3. Sol-Gel Encapsulation of Enzymes for High-Throughput Assays

As mentioned earlier, the encapsulation of enzymes within sol-gel materials for bioanalytical purposes has been extensively and continuously reviewed over the last 15 years or so. In terms of materials, recent evolutions are mostly dedicated to build up more complex multicomponent systems, especially for the design of enzymatic electrochemical biosensors [178], in a way that is very similar to immunosensors described in section 4.1. Evaluation of non-silica sol-gel materials [179], mesoporous particles [180] as well as novel composites involving ionic liquids [181] or incorporating whole cell extracts [182] as enzymatically-active systems was reported.

In parallel, the sol-gel encapsulation process was evaluated as an alternative and potentially superior method for current biotechnological applications of immobilized enzymes and especially high-throughput assays. The first attempt to design silica-immobilized biocatalysts arrays dates back from the works of Park and Clark in 2002 [183]. A PDMS film pierced with *ca*. 150 holes was deposited on a glass substrate and the cavities were filled a silica precursor solution containing the enzymes. After gelation, another pierced PDMS film was deposited and the enzyme substrates added. Colorimetric tests of activity could be measured for 20 different enzymes, mainly lipases and proteases. Later on, an alternative technological solution was proposed based on a pin-printing approach for the deposition of silica spots containing one or several enzymes [184,185]. Ultimately, commercially-available microarrayers were found well-adapted to the deposition of 525 sol-gel spots chips [186]. This approach was used to design microchips for drug screening. Human cytochrome P450 enzymes were encapsulated in sol-gel spots deposited on a glass slide and put in contact with a pro-drug. A cell monolayer prepared on a glass slide was then deposited on the top and stamped with the sol-gel array. After reaction, the array was removed and the cell viability determined by fluorescence imaging. Alternatively, P450-containing microsomes were immobilized within silica thin films formed in microassay plates [187]. Specific metabolites originating from drug bioconversion could be observed by fluorescent microscopy, offering the possibility to easily perform inhibitor assays. Encapsulation of enzymes in monolithic silica chromatography columns for inhibitor assays was also reported. In one example, the immobilized dihydrofolate reductase enzyme allowed the retention of inhibitors based on their bioaffinity for the protein [188]. In a following work, acetylcholinesterase (AChE) [189] was immobilized in capillary scale monolithic silica, allowing the identification of two enzyme inhibitors from 52 mixtures containing 1040 compounds.

Another area of application of immobilized enzymes is peptide mapping, based on the trypsin protease activity. Silica gels in multi-well plates [190] and silica films deposited on porous silica monoliths [191] were found suitable to high enzyme stability upon continuous use. Not surprisingly, the sol-gel encapsulation process was also combined with microfluidic devices by immobilization of trypsin in alumina/titania layers coating the microfluidic channels, leading to fast digestion rates [192]. This illustrates the versatility of the enzyme encapsulation sol-gel process and opens large perspectives for market development [193].

5. SOL-GEL ENCAPSULATION OF CELLS FOR BIOMOLECULE PRODUCTION

The production of biomolecules by sol-gel immobilized cells has attracted a great deal of attention because different types of cells from bacteria or fungus to plant or animal cells can be successfully employed for this purpose. However, there are several factors that must be taken into account before employing sol-gel immobilized cells in a bioprocess. For example: (i) as mentioned before each cell type would have specific immobilization requirements, (ii) the porosity of the materials is also important especially for high molecular weight proteins or enzyme production, (iii) the hydrophilic/hydrophobic properties of the materials which can determine the degree of adsorption of the product to the matrix. In this section, key examples of the employment of different types of cells for the production of a variety of biomolecules, from the laboratory to bioreactor scale are presented (Fig. (**5**) and Table **1**).

Among the multitude of methods for enhancement of cellular enzyme production, sol-gel encapsulation has proven to be a particularly easy and effective way to immobilize whole cells in order to obtain high thermal and long-term operational stability [194]. These enzymes can have different industrial applications; for example, the production of xylanase, an enzyme of great importance in the food and feed industries, by the fungal strain *Aspergillus awamori* K-1

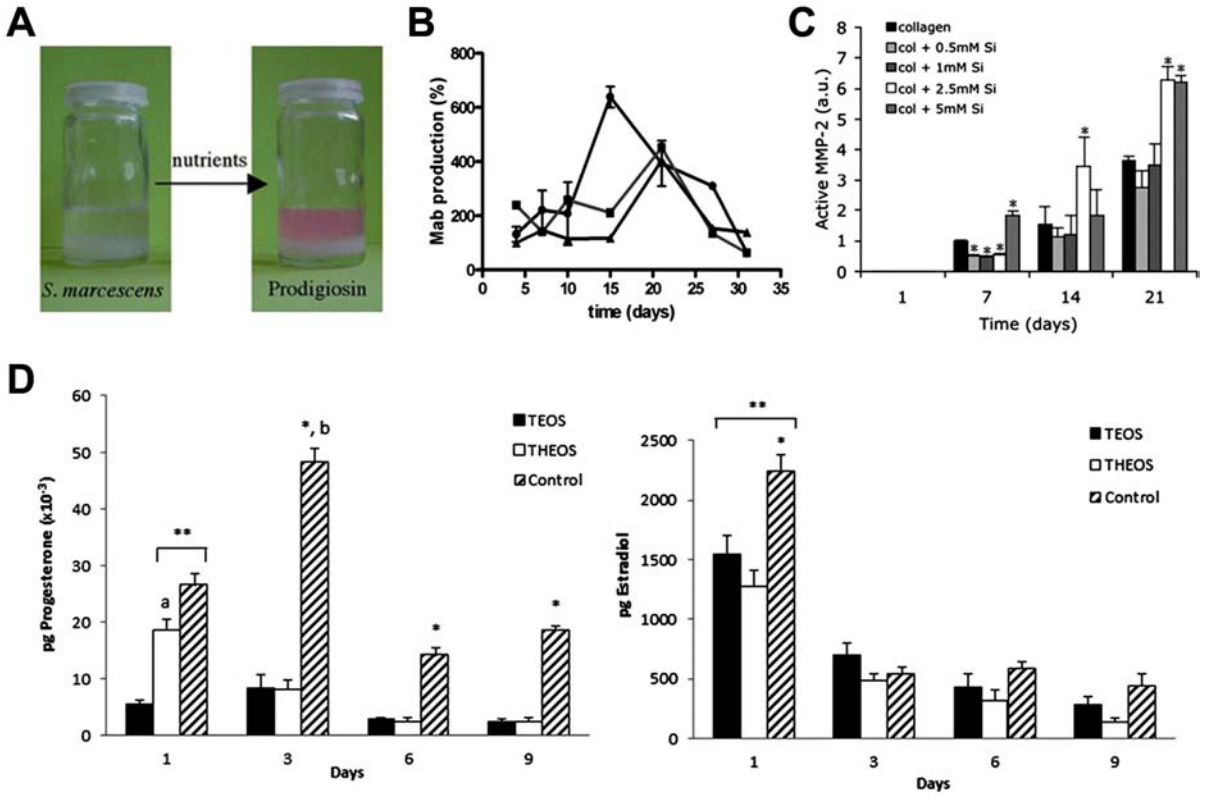

Fig. (5). Production of biomolecules by sol-gel immobilized cells: (**A**) production of prodigiosin by sol-gel immobilized *Serratia marcescens* [199]-Reproduced by permission of The Royal Society of Chemistry, (**B**) production of monoclonal antibodies by sol-gel immobilized hybridoma cells [32]-Reproduced by permission of The Royal Society of Chemistry, (**C**) production of MMP-2 by fibroblast immobilized in silicified collagen hydrogels [235]-Reproduced by permission of The Royal Society of Chemistry and (**D**) production of progesterone and estradiol by sol-gel immobilized ovarian follicles [229]-Reproduced by permission of The Royal Society of Chemistry.

cells immobilized in hybrid matrix composed of TEOS, 5% xylan and 15% calcium alginate was reported. The immobilized cells showed an increase in their capacity of xylanase production during continuous cultivation compared with free cell culture [195]. Food enzyme α-galactosidase produced by the fungus *Humicola lutea* immobilized in a hybrid sol-gel matrix, consisting of TEOS as a precursor and a mixture of PEG and PVA, under semi-continuous shake flask cultivation had a 2-fold higher enzyme activity compared to free cells [196, 197]. These enzymes are used for different biotechnological applications, e.g., for the hydrolysis of raffinose in beet sugar syrups or galactooligosaccharides in soybean milk. They are also of interest in medicine, e.g., for the treatment of Fabry's disease by enzyme replacement therapy or for the blood type conversion (B type cell to O type cell).

Besides enzyme production, immobilized microorganisms can be used for the synthesis of secondary metabolites. In recent years, secondary metabolites produced by microalgae have become increasingly economically important and have attracted the interest of researchers. Astaxanthin is a fat-soluble carotinoid pigment, and is mainly produced biologically by the microalgae *Haematococcus pluvialis.* Astaxanthin is used as a supplementary feed in fish farming and as a natural dyestuff in food and cosmetic industries. New biocer materials consisting of the living microalgae cells *H. pluvialis* immobilized within modified sol-gel silica layers can produce this metabolite in a continuous biotechnological process [198]. The encapsulation of *Serratia marcescens* in silica matrices containing glycerol was also studied for the production of a red pigment called prodigiosin that has promising therapeutic properties like antibacterial and cytotoxic effects [199].

Immobilized bacteria and mammalian cells can also be used for the production of biopharmaceuticals that include a variety of proteins such as recombinant proteins (blood factors, hormones, growth factors, interferons or interleukin-based products) and monoclonal antibodies. Some works can be found in literature involving protein production of biomedical interest from sol-gel immobilized cells. In this group we can include the synthesis of Interleukin-2 by Jurkat cells immobilized in alginate microspheres with a siliceous membrane [200] as well as the production of recombinant proteins from sol-gel immobilized *Escherichia coli.* In this respect, silicon oxide matrices have been successfully used for the long term preservation of transformed bacteria [201] and for the production of three different recombinant proteins: the chimerical TCR β chain expressed in the cytoplasm and the bacterial Sags Streptococcal Superantigen (SSA) and Staphylococcal Enterotoxin G (SEG) expressed both as soluble proteins in the periplasm which were fully biologically active [202]. Premkumar *et al.*, demonstrated encapsulation of recombinant *E. coli* which produce fluorescent proteins [203, 204]. The immobilization of hybridoma cells in

Table 1. Production of biomolecules by sol-gel immobilized cells.

Cell Type	Strains	Product of Interest	Uses	Refs.
Bacteria	*Serratia marcescens*	Prodigiosin	AntibacterialCytotoxic effects	[199]
	Escherichia coli	Chimerical TCR β chain	Molecules of the immune system involved in surface recognition of T-cells	[201, 202]
		SSA and SEG	Heat denaturation and protease resistant molecules absorbed as immunologically intact proteins by epithelial cells	
		Fluorescent proteins	Sensors	[203, 204]
Algae	*Haematococcus pluvialis*	Astaxanthin	Supplementary feed in fish farmingNatural dyestuff in food and cosmetics	[198]
Fungus	*Aspergillus awamori* K-1	Xylanase	Chlorine-free bleaching of wood pulpFood additiveIn combination with pectinase and cellulase for clarification of fruit juices and degumming of plant fiber sources	[195]
	Humicola lutea	α-galactosidases	Hydrolysis of raffinose in beet sugar syrupsTreatment of Fabry's disease by enzyme replacement therapy	[196, 197]
Mammal	Jurkat cells	Interleukin-2	Immunotherapy for the treatment of cancers, chronic viral infections and as adjuvants for vaccine	[200]
Plant	*Catharantus roseus*	vincristinevinblastine	Used in cancer chemotherapyAntimicrotubule drug	[205]
Hybridoma	Private clones 163B6	MAbs against cruzipain	Cysteine protease expressed by *Trypanosoma cruzi*	[32]
	Private clones 4C5A11	MAbs against human PGRP-Ia	Molecules that recognize peptidoglycan	

sol-gel derived silica matrices was also reported in different silica precursors and the viability of the cells as well as their monoclonal antibody (Mab) synthesis and release into the culture media was analyzed. It was observed in this case that THEOS was the preferred precursor for the immobilization of the cells in terms of viability and Mab release into the culture media [32].

Finally, the immobilization of plant cells for the production of alkaloids (vincristine and vinblastine) was accomplished by Carturan and coworkers. In this work, cells from *Catharantus roseus* were supported on a polyester fiber and exposed to a gaseous flux of silicon alkoxides, which formed a deposit of SiO_2 on the living cells surface upon reaction with H_2O adsorbed on the cellulose membrane of vegetable cells [205]. Viability and alkaloid production of cells were maintained after encapsulation; even more, secondary metabolite productivity was increased by two orders of magnitude with respect to that of free cells.

6. SOL-GEL ENCAPSULATION OF CELLS FOR BIOARTIFICIAL ORGANS AND TISSUE ENGINEERING

The interest in this field is based on the possibility of designing cell-based drug delivery systems and artificial organs in which transplanted cells could be protected from immune rejection avoiding the need of immunosuppression [206, 207]. Moreover, the biocompatible three-dimensional structure created around cells could mimic the *in vivo* environment preserving architecture, cell-cell and cell-matrix interactions in a better way than what can be achieved in two-dimensional culture systems [208, 209]. Cell-biomaterial interactions are largely governed by the stiffness and chemical composition of the substrate, although the surface topography of sol-gel based surfaces has also been shown to play an important role in the adhesion and proliferation of various cell types to different substrates [210, 211]. In this section, we emphasize key examples of 3D immobilization of mammalian cells in sol-gel matrices. Especially, we focus in these developments that require biocompatible synthesis procedures. In this sense, one can distinguished two main groups of mammalian cells that were successfully immobilized in sol-gel materials. On the one hand, the success of the sol-gel process in this field was initially related to the encapsulation of groups of cells and afterwards advances involving the development of novel synthetic procedures, biohybrids and functional nanocomposites extended the technology to individual cells. On the other hand, there are non-adherent or adherent cells with different requirements and especially different cell-material interactions. In the first case, the use of low concentration of silica precursors and addition of poly-alcohol molecules allow the successful immobilization of certain mammalian cells, such as hybridoma cells [32]. Meanwhile, for the adherent cells, the incorporation of specific biopolymers, such as collagen, to support cell adhesion is required (Fig. **6**).

6.1. Immobilization of Langerhans Islets for Bioartificial Pancreas Design

The first and most reported mammalian cells that were subjected to sol-gel immobilization for biomedical

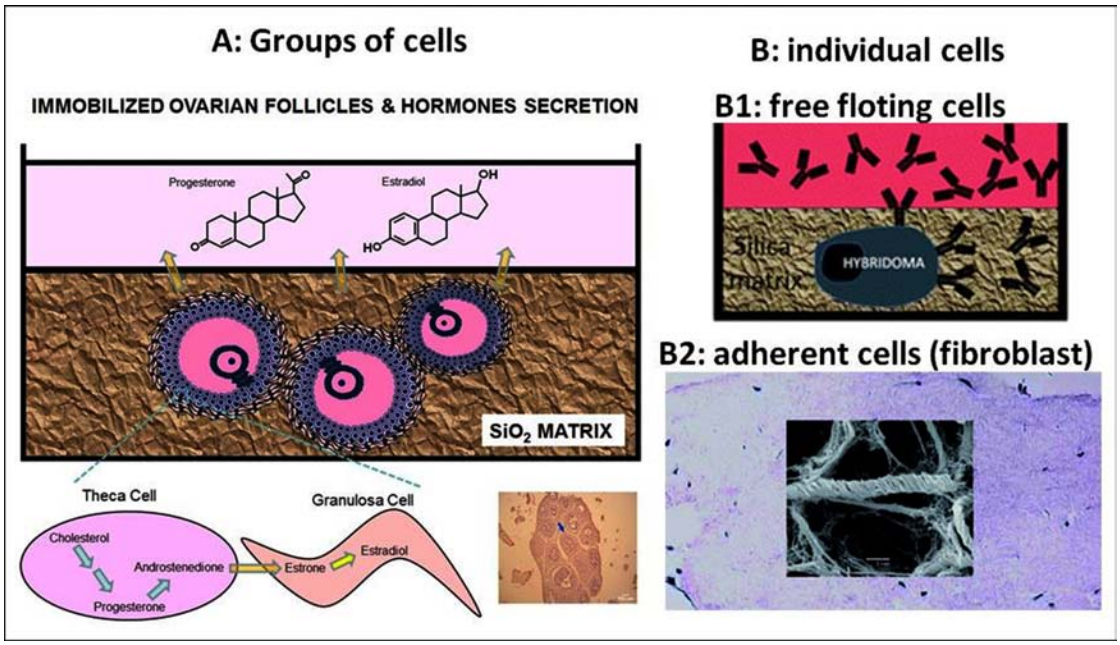

Fig. (6). Immobilization of cells: (**A**) groups of cells forming ovarian follicles [229]-Reproduced by permission of The Royal Society of Chemistry, (**B1**) non adherent hybridoma cells [32]-Reproduced by permission of The Royal Society of Chemistry, (**B2**) adherent fibroblast cells within collagen hydrogels.

applications were from the pancreas mainly because it is the organ involved in diabetes. The Islets of Langerhans are clusters of cells containing 3,000 to 4,000 cells. Within each islet are four different types of cells, which work together to regulate blood sugar level. The majority are insulin-producing beta cells and glucagon-producing alpha cells. Beta cells sense sugar in the blood and release the necessary amount of insulin to maintain normal glycemia. The loss of these cells makes the body incapable of producing insulin. For example, in type I diabetes the immune system recognizes beta cells as foreign bodies and destroys them. Although insulin-producing islet cells cease to function in persons with diabetes, the remaining 98 % of the pancreas continues to function normally, producing digestive enzymes. Transplantation of immobilized pancreas islets has been suggested as a method to treat this disease because it requires no immunosuppressive therapy and no regular self-injection of insulin.

In 1997, Pope *et al.*, were the pioneers in reporting the sol-gel immobilization of viable animal cells, i.e. Langerhans islets. *In vitro* studies have proven that sol-gel encapsulated mouse Langerhans islets can produce and secrete insulin. Furthermore, encapsulated islets were transplanted interperitoneally in several diabetic mice and were proven to significantly lower their urinary sugar content for 11 weeks following silica gel-encapsulated Langerhans islet transplantation [212, 213].

These preliminary results were followed by Carturan and co-workers who, in a series of publications, demonstrated that pancreatic rat islets would be encapsulated by a siliceous layer deposited on the surface of single islets upon reaction with gaseous siliceous precursors employing the so-called Biosil process [214, 215]. The characteristics and thickness of the siliceous layer can be tailored in order to allow the diffusion of insulin without altering the structure, viability and function of the cells of the Langerhans islets [216]. These new methods take advantage of the islet capsule surface as a template for silica formation. Alternatively, mouse and human islets were recently exposed to medium containing saturating silicic acid levels for 9-15 min. The resulting pancreatic islets surrounded by porous sílica were able to survive and accomplish their function [217].

Sakai *et al.*, developed an alternative type of sol-gel based immunoisolation membrane for bioartificial pancreas. The alginate/aminopropyl-silicate/alginate membrane could be tune in order to successfully rejected γ-globulin, while retaining good permeability to substances of low molecular weight [218, 219]. Islets could be encapsulated within the membrane maintaining their viability and insulin secretion function [220-222]. Rat islets encapsulated in the membrane were transplanted to the peritoneal cavities of diabetic mice that presented normoglycemia for 105 days after transplantation without administration of immunosuppressive drugs [223]. Particularly, not only the primary cells but also genetically-engineered cells, like the pancreatic β-cell line (MIN6) cells, have been encapsulated expanding the applicability of sol-gel immobilization to treat a wide variety of diseases [224].

6.2. Bioartificial Organ Capable of Supporting Hepatic Function

Liver failure is a life-threatening condition that demands urgent medical care. Liver failure can occur gradually and over many years being various the causes of chronic liver failure (*i.e.* the long term alcohol consumption in high quantities or virus infections). In addition, acute liver failure occurs rapidly and can be associated to different factors including virus infection such as hepatitis. Thus, for patients with

liver failure it would be highly desirable to have a bioartificial organ capable of supporting hepatic function. Especially, hepatocytes are the functional cells of the liver and perform several metabolic, endocrine and secretory functions. The Biosil process was identified as an effective alternative to develop bioartifical liver devices. Indeed, entrapped hepatocytes cultured in collagen gels and covered by a homogeneous silica layer produced bilirubin monoconjugate, removed ammonia, synthesized urea and produced diazepam metabolites in a similar level than controls hepatocytes [225, 226].

More recently, the encapsulation of hepatocellular carcinoma cell line (HepG2) within resistant mineralised beads composed of an alginate-silica core and an external Ca-alginate layer was reported. The preliminary results showed that entrapped cells can be kept alive for at least 6 weeks post-encapsulation [227]. While, *in vivo* experiments revealed the biocompatibility of the material [228].

6.3. Development of Ovarian Follicles Cell-Based Hormone Delivery Systems

There are many diseases that require a treatment whose effectiveness depends on hormone administration. Particularly, the hormone replacement therapy is the treatment used to relieve symptoms associated with menopause in women or to treat Turner syndrome. However it was reported that oral administration of estrogen causes hypertriglyceridemia and increased risk of gallbladder disease and thromboembolism. In this context and in view of the drawbacks of current treatments, the search for new therapies that can guarantee a physiological administration of hormones such as ovarian cell-based drug delivery systems is a key to improve treatments and minimize adverse effects. Ovarian follicles are formed by different cell types, the most important being the external theca cells which produce progesterone and the internal granulosa cells which employ progesterone to produce estradiol. Encapsulation of ovarian follicle cells within silica matrices was recently reported [229]. It was shown that rat ovarian follicles encapsulated in silica-based matrices remain viable and retain the cellular structure and steroid secretion function *in vitro*. It was hypothesized that the immobilization process would affect primarily the external theca cells, which are in close contact with the silica matrix and sol-gel by-products and to a lower extent the internal granulosa cells. However, the collaboration between the two mammalian cell groups in the production of estradiol within a sol-gel silica matrix was demonstrated.

6.4. Human Umbilical Vein Endothelial Cells (HUVEC)

Snyder *et al.*, reported how aqueous sols of highly monodispersed, size-tunable and stable silica nanoparticles in the presence of the basic amino acid L-Lysine (called Lys-Sil sols) can be harnessed as a precursor medium for cell encapsulation [230, 231]. Human umbilical vein endothelial cells (HUVEC) were encapsulated by dispersion in growth media containing Lys-Sil nanoparticles, the assembly of which was triggered by titration with di-lysine. The cytocompatible conditions of the Lys-Sil sol formulation promoted the sustained viability of the encapsulated mammalian cells [232]. However, the lack of *in vivo* evaluation of these materials does not allow to conclude on their usefulness.

6.5. Skin Tissue Engineering

The question is raised whether other adhering cells of high biomedical interest, such as fibroblasts or epithelial cells, can also be immobilized within sol-gel based materials, targeting applications in tissue engineering. Earlier reports using the Biosil process showed that 3T3 mouse fibroblasts could be encapsulated provided their pre-immobilization in biopolymer scaffold [233]. Later on, attempts were made to design an encapsulation protocol based on silica only. A modified two-step sol-gel route using a first low pH hydrolysis step of TEOS followed by a neutral condensation-gelation step has been successfully used as a cell encapsulation matrix for 3T3 mouse fibroblasts and CRL-2595 epithelial cells [234]. A high water-to-TEOS ratio and the addition of D-glucose as a porogen and source of nutrients were chosen to minimize silica dissolution and improve the biocompatibility of the process. However, individual fibroblasts did not extend in a 3D network, remaining round-shaped. This suggests a poor adhesion of the cells with the silica surface that was evidenced by a rapid cell death post-encapsulation. Interestingly, the viability of the cells was significantly improved when groups of cells were formed prior to their addition to the silica sol.

More recently, a biocompatible procedure that allows the simultaneous self-assembly of collagen and polymerization of aqueous silicates in the presence of cells was reported and successfully applied to the 3D immobilization of fibroblasts [235]. However the highest survival rate was for hybrid materials obtained in the presence of low sodium silicate concentration (1 mM) and the lower one was with 5 mM sodium silicate concentration. Silica-collagen bionanocomposite hydrogels were also obtained by addition of silica nanoparticles to a collagen suspension followed by neutralization. These bionanocomposite materials showed lower surface contraction and higher viability of entrapped cells [236]. An increase in collagen concentration allowed the incorporation of higher amounts of silica [237]. Overall, it was observed that silicification significantly improved the mechanical and thermal stability of the collagen network within the hybrid systems, while nanocomposites were found to favor the metabolic activity of immobilized human dermal fibroblasts while decreasing the hydrogel contraction. Moreover, *in vivo* implantation in subcutaneous sites of rats confirmed significant fibroblast colonization and endothelial cells organization in open tubular structures while the infiltration of macrophages was very little [238]. This opens large perspectives to use hybrid and nanocomposite silica-collagen materials as biological dressings [239].

CONCLUDING REMARKS

This review has no claim for being exhaustive in presenting the achievements of the sol-gel bioencapsulation technology. However, it was prepared so as to present enough examples from the literature to provide a complete overview of the fields relevant to medicinal sciences where the sol-gel process can be envisioned as an alternative, if not a better, solution compared with the (bio)-polymer technology to achieve three-dimensional bio-immobilization in a way that combines a robust material with the long-term preservation of the biological function. From a chemical point of view,

the versatility of the sol-gel technology is almost unlimited in terms of composition and shape. Basic recipes can be found in the literature and can be reproduced with modest practical skills. Yet the large amount of publications describing slightly different protocols together with the fact that sol-gel chemistry is not taught in current organic chemistry classes still makes this technology rather exotic for a large part of the life science community. In parallel, many questions about the biochemistry, cytotoxicity, biodegradation and biocompatibility of sol-gel based metal oxides are still pending. The future of this field is therefore in the hands of pluridisciplinary partnerships.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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