

Sol-gel technology for biomedical engineering

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Abstract. Sol-gel derived silica possess many promising features, including low-temperature preparation procedure, porosity, chemical and physical stability. Applications exploiting porous materials to encapsulate sensor molecules, enzymes and many other compounds, are developing rapidly. In this paper some potential applications, with emphasis on biomedical and environmental ones, are reviewed. The material preparation procedure is described and practical remarks on silica-based sol-gels are included. It is reported that sol-gels with entrapped various molecules may be used in construction of implants and coatings with bioactive properties. It is shown how to exploit the sol-gel production route for construction of sol-gel coated fiberoptic applicators for lasertherapy. The applications of bioactive materials are discussed, as well. It is demonstrated that it is possible to immobilize photosensitive compounds in sol-gel matrix without losing their photoactivity. Some examples of sol-gel based biosensors are demonstrated, as well, showing their potential for detecting various gases, toxic substances, acidity, humidity, enzymes and biologically active agents.

Key words: sol-gels, biosensors, bioactive coatings, biomedical applications.

1. Introduction

A sol-gel method enables the powderless processing of glasses, ceramics and thin films or fibers, directly from solution. Precursors are mixed at the molecular level and variously shaped materials may be formed at much lower temperatures than it is possible by traditional methods of preparation. One of the major advantages of sol-gel processing is the possibility to synthesize hybrid organic-inorganic materials. Combination of inorganic and organic networks facilitates the design of new engineering materials with exciting properties for a wide range of applications.

The pioneering works on those materials were led by Schmidt [1] and Wilkes [2]. They had investigated the possibilities of changing the properties of sol-gel derived materials by incorporating the organic components to a silica matrix. Organic-inorganic hybrids have been called ORMOSILS (ORganically MODified SILicates), ORMOCERS (ORganically MODified CERAMics) and CERAMERS (CERamic polyMERS) or POLYCERAM (POLYmeric CERAMics). Hybrid materials offer the opportunity to combine the desirable properties of organic polymers (toughness, elasticity) with those of inorganic solids (hardness, chemical resistance).

The first paper on sol-gels was published over 150 years ago by Ebelmen [3], however, the rapid development of this technology and applications are observed from the last decades. The broad range of possible applications of sol-gel derived materials and biomaterials marks this technology as one of the most promising fields of contemporary material sciences [4–9].

2. Silica sol-gels

Materials produced in a sol-gel process, possess a wide range of physical attributes, the nature of which depends on the type

and amount of substrate used and other variables (including the preparation conditions like pH, temperature etc.).

The sol-gel technology based on various alkoxides, allows production of classical silica glasses, as well as multicomponent materials, merging silicates with titanates, borates and a variety of other oxides (Zn, La, Al, Li, B, K, etc.). The alkoxide gel method can be also used for production of certain non-silicate oxide glass-like materials (e.g., ZrO_2 , etc.) [10].

The organic-inorganic hybrid materials may be prepared in various ways. The simplest one relies on dissolution of organic molecules in a liquid sol-gel [11–16]. The other way uses the impregnation of a porous gel in the organic solution [17]. In the third type, the inorganic precursor either already has an organic group or reactions occur in a liquid solution to form chemical bonds in the hybrid gel.

The sol-gel process itself leads to formation of gels from mixtures of liquid reagents (sols) at ambient temperatures. It involves several steps: the evolution of inorganic networks, formation of colloidal suspension (sol) and gelation of the sol to form a network in a continuous liquid phase (gel). Drying of the obtained gels, even at room temperature, produces glass-like materials called xerogels (xeros – dry).

The popular sol-gels are silica based sol-gel materials. The production process comprises several steps. First, silicate precursor (e.g. tetraethylortosilicate TEOS or tetramethylsilicate TMOS) is mixed with solvent and catalyst and stirred for a few hours. This process leads to hydrolysis of the Si–O–R bonds. The hydrolysis reaction can be catalyzed by acids (HCl, HF etc.) or bases (NH_4OH , NaOH etc.). The hydrolysis, gelation and aging/drying processes occur simultaneously. During the “aging” step (after gelation and before drying) the sol-gel derived material expulses the liquid phase (solvent which can be water or alcohol) in the process called syneresis.

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Pronounced drying at temperatures not exceeding 100°C leads to formation of “dry” gels, called xerogels. They are relatively sturdy, typically transparent but porous materials. The pore size depends on such factors as time and temperature of the hydrolysis and the kind of catalyst used. The diameter of the pores is directly related to the shrinkage of the “wet” gels. During the drying process the gel volume decreases even several times (which is the main reason of cracking). Addition to the hydrolyzate of small amounts of simple organic solvents (e.g., dimethylformamide or dimethylsulfoxide), stabilizes the resultant gels increasing their mechanical strength and improving transparency.

The whole process of forming sol-gels is schematically presented in Fig. 1.

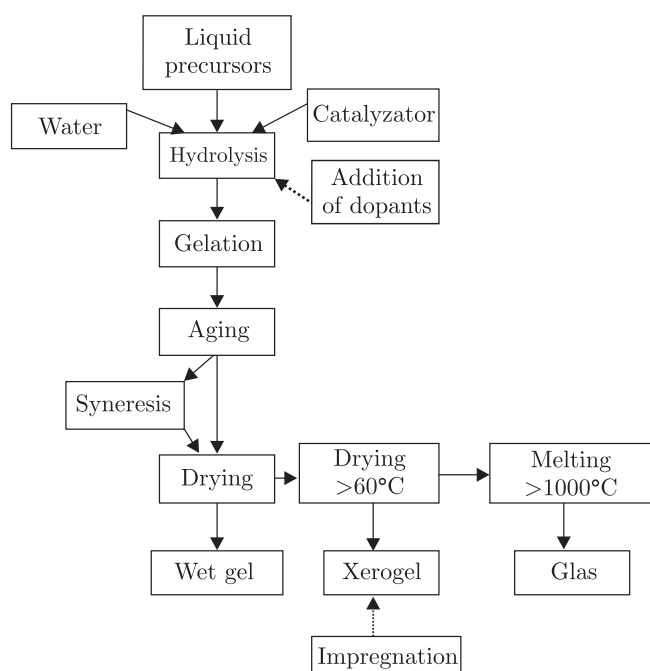


Fig. 1. The schematic representation of the sol-gel production process

3. Sol-gel coatings and implants

3.1. Coatings applications. Various materials may be coated with liquid sol-gel hydrolyzate, thus changing their characteristics [18–21], preventing corrosion [22], improving biocompatibility [23] or electrical insulating properties [24]. The sol-gel coatings properties are the subject of many examinations [25–28].

In our group silica based sol-gel coating are used for production of fiberoptic applicators for laser therapies [29]. Interstitial laser thermotherapy is quite new treatment modality designed for minimal invasive destruction of pathologic tissues in difficult to access environments (e.g. brain, liver). Fiberoptics laser applicators are used to perform interstitial therapy with laser light, whereas the applicator is inserted into the pathologic lesion and curing laser light is guided through the fiber [30,31]. We already demonstrated that sol-gel coatings may improve the characteristics of applicators. Depending on

the amount of compounds used for producing sol-gels different optical and structural properties could be achieved [32–34]. Figure 2 shows light distribution around the sol-gel coated applicator. The applicator was made by dip-coating method, where the bar fiber (2,5 cm long tip, core, no jacket and coat) where placed in liquid hydrolyzate.

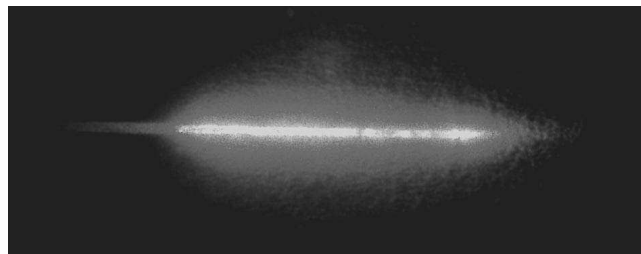


Fig. 2. Light distribution from the sol-gel coated fiberoptic applicator

3.2. Sol-gel supported implants. Devices and prostheses made from biomaterials should last without failure for lifetime of the patient. The challenge is that the lifetime of patients has progressively increased during XX century up to 80+ years. Considerable attention has been directed towards use of bioactive fixation of implants, where term “bioactive fixation” is defined as interfacial bonding of an implant to tissue by means of formation of a biologically active hydroxylapatite layer on the implant surface [35]. An important advantage of bioactive fixation is that a bioactive bond to bone has strength equal to or even greater than bone after 3–6 months. The high strength of both hard and soft tissue bonding to bioactive implants is due to in vivo growth of a dense layer of nanometer-scale hydroxycarbonate apatite crystal agglomerates, which bind to collagen fibrils. Comparative studies of various compositions of bioactive glasses, ceramics and glass-ceramics show that there is a considerable range of bone bonding to bulk implants or rate of bone proliferation in the presence of bioactive particles. However, the emphasis in scientific investigations should be shift from replacement of tissues to a new concept of tissues regeneration supported by use of materials activating the body’s own repair mechanisms. This idea combines osteogenesis and chondrogenesis at a molecular biological level with the molecular design of a new generation of bioactive materials that stimulate proliferation and differentiation of osteoprogenitor cells and enhance rapid formation of extracellular matrix.

Bacterial infection due to an implanted medical device is a potentially serious complication, typically leading to premature implant removal, which is costly, traumatic to the patient and might be lethal. For that reason nitric oxide-releasing sol-gels were examined as antibacterial coatings for orthopaedic applications using medical-grade stainless steel [36]. The antibacterial effectiveness of these coatings was evaluated with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The bacterial adhesion resistance of NO-releasing coatings was studied in vitro by exposing bare steel, sol-gel, and NO-releasing sol-gel-coated steel to cell suspensions. Cell adhesion to bare and sol-gel coated steel was

similar, while NO-releasing sol-gel surfaces had significantly less bacterial adhesion for all species and temperatures investigated.

To improve biocompatibility of titanium, hydroxyapatite and fluor-hydroxyapatite sol-gel layers were deposited on titanium substrate [37]. The obtained on titanium discs sol-gel films were uniform and dense, with a thickness 5 μm. The dissolution rate of the coating layer decreased with increasing fluor ions incorporation within apatite structure, which demonstrates the possibility of tailoring the solubility by a functional gradient coating of hydroxyapatite and fluor-hydroxyapatite. The alkaline phosphatase activity of the cells (osteoblast-like MG63 line and human osteosarcoma HOS cell line) on all the hydroxyapatite and fluor-hydroxyapatite coated samples showed much higher expression levels compared to pure Ti. This confirmed the improved activity of cell functions on the substrates with the sol-gel treatment.

Two methods for forming thin, crack-free calcium phosphate films on Ti6Al4V implants with sintered porous surface regions were described [38]. Thin films were formed on implants as an approach to increasing the rate of bone ingrowth. The films were prepared using either inorganic precursor solution or an inorganic precursor solution. Both resulted in the formation of nanocrystalline carbonated hydroxyapatite films, but with different Ca/P ratios and structures. The inorganic route-formed films displayed more irregular surface texture and were less dense. Both developed coating surface topographies suggested to be good model systems for further in vivo studies to differentiate the effect of surface topography and surface chemistry on osteoconductivity and osteointegration potential of bone-interfacing implants.

4. Bioactive sol-gel materials

The described in literature results demonstrate that the sol-gel technique is a versatile method for embedding and immobilizing bioactive compounds within an inorganic oxide matrix and for the preparation of new bioactive coatings [39]. The sol-gel matrices can be transparent, inert, non-toxic, thermally stable, and forms stable coatings on such varied substrates as polymer foils, paper, tissue, metal or wood. Oily and high viscosity substances of pharmaceutical interest can also be incorporated without problems.

The concept of biocompatible, osteoconductive material mimicking the structure of natural bone to be used as bone graft is very promising. For this purpose it is possible to apply the sol-gel technology [40]. The authors reported on a novel and simple sol-gel technique for fabricating pure hydroxyapatite powders. Good reproducibility, high yield, simplicity of execution, not use of solvent except water made described synthesis very competitive related to other wet and sol-gel processes and suitable for being scaled up to industrial size.

Bioglasses are important bioactive materials and have been used for the repair and reconstruction of diseased bone tissues, as they exhibit direct bonding with human bone tissues [41]. However, bioglasses have usually low mechanical properties and thus are used as coatings on stronger substrates. The 58S

bioglass powder ($\text{SiO}_2\text{-CaO-P}_2\text{O}_5$) was produced by sol-gel method and then pressed into the discs sintered at 500, 800, 1000 and 1200°C. The apparent density and the Vicker's hardness of the sintered bioglass were tested using densitometer and a Vicker's hardness tester, the thermal properties were studied using differential scanning calorimetry and dilatometry, and the crystallization of the bioglass was examined by means of X-ray diffraction and scanning electron microscopy. The sintered bioglass discs were then immersed in the simulated body fluid solution. The bioglass surface after the immersion was examined using field emission SEM. The formation of apatite layer was confirmed using Fourier transform infrared spectroscopy. A dense apatite layer was formed on the highly bioactive amorphous bioglass, whereas incomplete or porous apatite layer was formed on the bioglass subjected to crystallization. At sintering temperatures above 800°C crystalline phase CaSiO_3 was formed, what caused decrease of bioactivity of the bioglass.

Three dimensional hierarchical structure composed of macropores and micropores was created via sol-gel route [42]. The resultant macroporous bioglasses exhibited excellent mechanical properties. Moreover, the large pores and high porosities were conducive to tissue in-growth and nutrient delivery to the center of the regenerated tissues. The improved mechanical strength, the good apatite-forming ability confirmed by in vitro tests and the high specific area make these materials candidates for tissue repair and tissue engineering applications.

Sol-gel technique requires no expensive apparatus, is flexible in terms of substrate geometry, and can be exploited for various coatings. Sol-gel processing was used to coat titanium substrates with hydroxyapatite, TiO_2 , and poly(DL-lactic/glycolic acid) [43]. Coated surfaces were analyzed with XRD, EDS, AFM, SEM, and water contact angle measurements. It was demonstrated that these coatings possess a high degree of crystallinity and good resistance to cracking. Crack-free coatings had micron-range surface roughness with islands ($< 10 \mu\text{m}$). Sol-gel layers were also evaluated by cytocompatibility testing with osteoblast-like cells or bone-forming cells. Results of the cytocompatibility tests showed that osteoblast-like cell adhesion was promoted on the novel hydroxyapatite sol-gel coating compared to traditional plasma-sprayed hydroxyapatite coating. Moreover, hydrothermal treatment of the sol-gel coating improved osteoblast-like cell adhesion. Since osteoblast adhesion is a necessary prerequisite for subsequent formation of bone, these results provided evidence that hydrothermally sol-gel processed hydroxyapatite may improve bonding of titanium implants to juxtaposed bone.

The surface of biomedical metallic implants covered by a bioactive apatite film can create bioactivity of the implant and shorten healing time [44]. Apatite films on Ti6Al4V were prepared by sol-gel route using $\text{Ca}(\text{NO}_3)_2$, P_2O_5 and HPF_6 as the precursors. In vitro evaluations of the resulting hydroxyapatite and fluorapatite/hydroxyapatite solid solution films were done in simulated body fluid and citric acid modified phosphate buffer solution. The fluorapatite/hydroxyapatite film demonstrated to have good bioactivity, and to have better stability in citric acid modified phosphate buffer solution and higher adhe-

sion strength than the hydroxyapatite film. When fluorine was incorporated into the film, an increase in crystallinity of the apatite film and a decrease in intrinsic solubility of the fluorapatite/hydroxyapatite film could make significant contributions to the improvement of the stability.

Biodegradable silica fibers were prepared from silica sols by dry spinning [45]. The spinnability of the sols and its influence on the fiber structure were investigated. The same sols can be used to prepare different fiber structure depending on the process stage, temperature and viscosity. The spinning moment was found to be important in controlling the biodegradation. Influence of catalysts (acid HNO_3 and/or base NH_3) as well as evaporation of the liquid on the process were investigated, as well. They did not have an influence on the spinnability, but they reduced the overall reaction time. The prepared samples were aged for 1 and 3 months indicating stable structure as a function of ageing time according to the biodegradation experiments, except in the case of high catalyst concentration. A porous structure was revealed using TEM. Heat-treatment of the fibers at temperatures below 300°C induced remarkable differences in the fiber bulk structure according to FT-IR measurements. By combining the concentrations of substrates, process stage, reaction circumstances and the viscosity of the sol properly, different fibers with adjustable biodegradation ma be prepared.

The sol-gel spin-coating process showed to be suitable tool for the deposition of CaTiO_3 coatings [46]. The composition

and structure of these coatings depended closely on the applied sintering temperature. An important activation of the Ti-O-Ca network condensation and crystallization was produced after sintering at 800°C . Films sintered over this transition temperature became polycrystalline and exhibited a perovskite structure. Described films presented a more compact aspect due to completion of the polycondensation-densification process. The less compacted films (i. e. sintered at 300°C) suffered from intense stresses during immersion in SBF, which led to cracked surfaces that did not appeared on the denser coatings. The experiments confirmed morphological and structural suitability of Ti-O-Ca coatings for their use as buffer layers between a TiAlV prosthetic alloy and a hydroxyapatite bio-ceramic outlayer.

A new completely soluble in water precursor tetrakis(2-hydroxyethyl) orthosilicate THEOS was used to synthesize monolithic hybrid materials on the basis of silica and 3 main types of carrageenans, κ -, ι -, and λ - carrageenans [47]. Carrageenans introduced into the precursor solution made use of common catalysts unneeded to trigger the sol-gel transition. The material properties were controlled by both, the precursor and carrageenan. The increase of silicate concentration led to a rise in the stiffness and brittleness of the material, whereas the polysaccharide addition made it softer and more elastic. Polysaccharides modified the structure of silica-based materials, because they transformed a 3D network of connected silica particles into that consisting of crossed fibers.

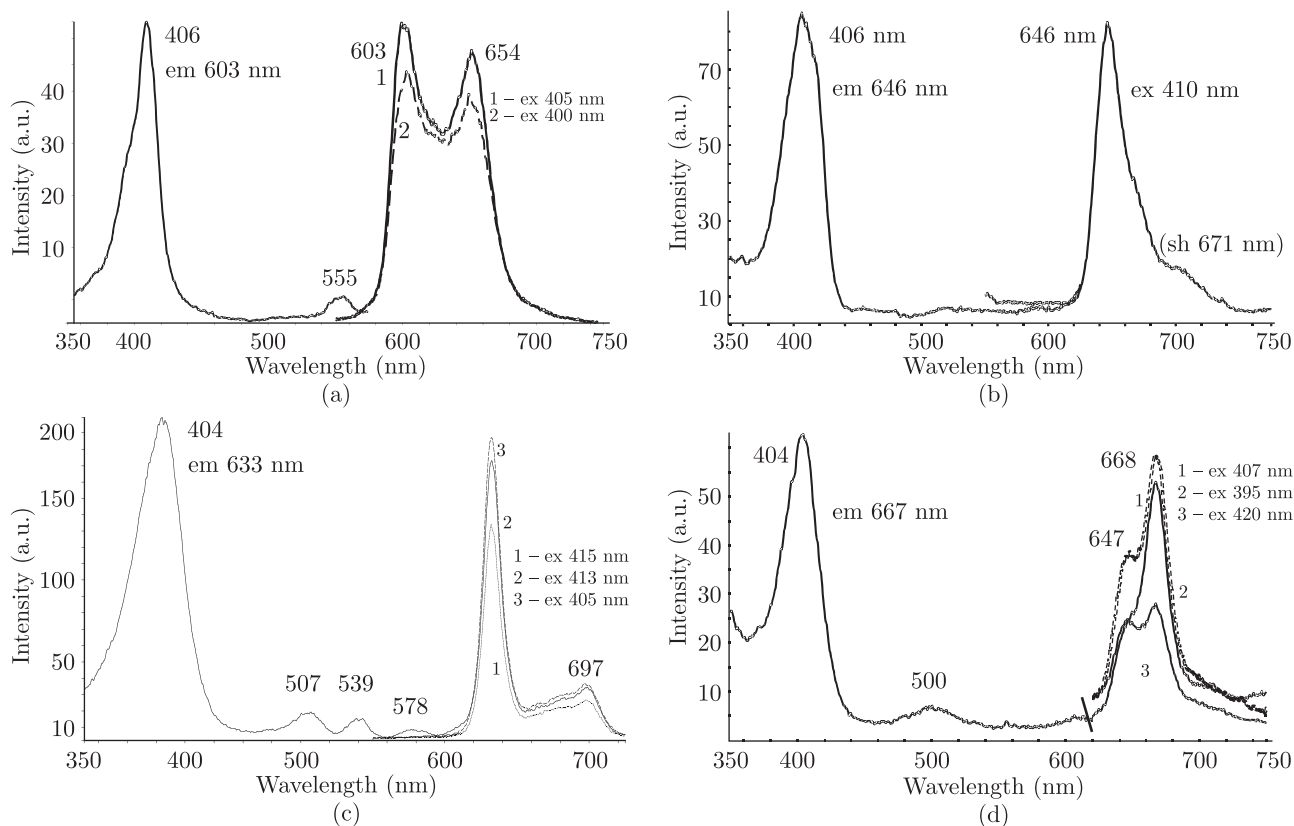


Fig. 3. Excitation spectrum (left) and emission spectra (right) from fiber optic applicators coated with silica sol-gel doped with photosensitive dyes. (a) applicator in air, doped with 1.69×10^{-4} M PP IX, (b) applicator in air, doped with 3.8×10^{-5} M Photolon, (c) applicator doped with PP IX, placed in methanol, (d) applicator doped with Photolon, placed in methanol

Self-assembling organic/inorganic sol-gel systems were used to prepare mesoporous silica coatings that can qualify for medical applications [48]. The cationic surfactant cetyltrimethyl ammonium chloride or an amphiphilic triblock copolymer were utilized as templates or structure-directing agents, and TEOS as the silica precursor. Thin sol-gel films were deposited on various substrates (glass, silicon, and titanium) by spin casting. Gel formation time equal 60s resulted in the optimum mesostructures. The use of organic templates permitted modification of the pore sizes of the mesochannels in the mesoscopic regime. The removal of the template to hollow the pores was accomplished by photocalcination (selective UV irradiation). The mesoporous structure and the presence of silanol groups was responsible for the capacity of these pure silica coatings to form apatite when soaked in SBF.

In our group the investigations are carried out on interstitial photodynamic therapy (PDT), where we propose to entrap the photosensitive compounds in the silica based sol-gel coatings, so they could react with the external environment [49]. The work is focused on the examination of optical properties of silica sol-gel biocoatings doped with porphyrine derivative PPIX and chlorophyll derivative Photolon. We demonstrated that these agents entrapped in sol-gel preserves their chemical activities and have contact with the external environment. Moreover, the some chemical reactions (e.g. with zinc cations and pyridine, as well as protonation) occurred quite fast. This indicates, that the interconnected porous network could be easily penetrated by relatively large molecules (e.g. mentioned pyridine molecule), whereas the sensitizers do not leave matrix pores. We also demonstrated that excitation is observed for sol-gel coated applicators occurs in air, as well as in hazardous environment, like e.g. in methanol. Some exemplary spectra are demonstrated in Fig. 3.

5. Bioencapsulation within sol-gels

Many biological compounds encapsulated within sol-gel matrices may serve as analytical devices for biocatalysis and biosensing, and as slow release systems, as well [50]. The revolution in the area of sol-gel-derived materials started since the demonstration that these materials may be used to encapsulate biological species such as enzymes, antibodies and other proteins in a functional state. Typical applications of sol-gel biomaterials include selective coatings for optical and electrochemical sensors and biosensors, stationary phases for affinity chromatography, immunoadsorbent and solid-phase extraction materials, controlled release agents, solid-phase biosynthesis, and unique matrices for biophysical studies.

The development of an immune process has allowed the in-situ observation of human cells in titania sol-gels [51]. The investigation technique was based on the use of an auto-immune reaction combined with a fluorescent agent that allowed a direct inspection of the cell behavior (human chondrosarcoma cells and pluripotential mesenchymal stem cells from bone marrow). After the in-vitro observations authors concluded that both TiO₂ and TiN surfaces showed enhanced biological responses (high level of cells adhesion, growth and proliferation).

Sol-gel derived mesoporous biomaterials were used in the flow-injection fluorescence immunoassay system [52]. Anti-gentamicin antibody was immobilized in a mesoporous sol-gel material using tetramethoxysilane as a precursor and poly(ethylene glycol) as a template. The sol-gel glass was used to develop an immunoaffinity column for the flow-injection immunoassay of gentamicin. The immunoassay was based on the competition between gentamicin and fluorescein isothiocyanate-labeled gentamicin for a limited number of encapsulated antibody binding sites. NaOH solution was used for regeneration of encapsulated antibody binding sites after each measurement, which allowed the immunoreactor to be used for up to 20 times without any loss of reactivity. It can be conclude that described method of biochemical analysis seems to be simple, rapid, stable, sensitive, and renewable.

Room temperature processed silica xerogels were examined as controlled release carriers for proteins [53]. Tripsin inhibitor was chosen as the model protein, because its size (21 kD) is similar to that of bone growth factors. The viability of using sol-gels intended to serve as both substrates for bone growth as well as to allow incorporated proteins such as growth factors to diffuse out and stimulate cell function and tissue healing was under investigation. The data documented that the in vitro release of tripsin inhibitor was dose and time dependent during immersion up to 9 weeks. The release pattern included an initially slow release, with further release occurring at a rate which was proportionate to the square root of time, an indicative of a diffusion time. All data taken together suggested that the xerogel materials are characterized by an excellent set of properties useful in bone repair applications.

First use of new precursor – tetrakis(2-hydroxyethyl) orthosilicate THEOS – for preparation of hybrid nanocomposites containing various polysaccharides and enzymes was reported by scientists from Russia [54]. Two different types of *O*-glycoside hydrolyses were taken for the immobilization. To reveal whether the polysaccharide inside the hybrid material influences the enzyme entrapment and functioning, negatively charged xanthan, cationic derivative of hydroxyethylcellulose and uncharged locust bean gum were examined. The amount of immobilized enzymes was small, comparable to their concentration in the living cells. It was shown by the scanning electron microscopy that the hybrid nanocomposites are sufficiently porous that allows the enzymatic substrates and products diffuse from an external aqueous solution, whereas protein molecules were immobilized firmly and not easily washed out of the silica matrix. A sharp increase of the enzyme lifetime (more than a hundred times) was observed after the immobilization.

The experiments with bacteria *Escherichia coli* cells showed that they can be entrapped within silica sol-gel matrices [55]. The cellular organization of these bacteria appears to be well preserved. Their β -galactosidase activity was measured via the hydrolysis of *p*-NPG. The formation of *p*-nitrophenol was followed by optical absorption. The enzymatic activity of concentrated *E. coli* suspensions was even improved by encapsulation. This might be due to the fact that sol-gel encapsulation prevents the agglomeration of bacteria

than remain randomly dispersed in the silica matrix. The water content of silica gels appeared to be a major parameter. The enzymatic activity of bacteria was preserved in wet gels even when the gel was aged at room temperature for 1 week, but decreased rapidly when the gel was dried under ambient condition. Much better results were obtained upon freeze drying, where 30% of the enzymatic activity was still observed.

6. Medical and environmental applications of sol-gel based sensing devices

6.1. Sol-gel optodes for fiberoptic sensors. Construction of the optode for optical biosensor requires immobilization of sensitive compounds in the host matrix. There are several methods enabling molecules entrapment. One can use gels, polymers, saccharose, various meshes and membranes [56]. In case of fiberoptic indirect sensors optode must be attached to the fiber tip. Nowadays, there are two commonly used optode host materials: sol-gels and polymers.

Silica gels seem to be good materials for construction of optodes for indirect fiberoptic sensors. Their visible transparency, porosity enabling the transport of gases or liquids through the material, thermal and chemical stability, and ability to be filled with additional active phases are the key properties that gels bring to sensor applications [57,58]. The sol-gel process, which leads to the silica glass formation, is carried out at room temperature what gives a wide range of potential applications. In addition, it is easy to prepare the sol-gel elements of various shapes.

Various indirect optical sensors and their applications are described in literature [59]. The optode can work as a chemical sensor that detects certain analytes in aqueous solutions or in air on chemical way. It means that changes in the environment cause the changes in the photosensitive material, which is immobilized in the optode matrix. These chemical changes influence the observed light intensity (for example, due to absorption) or one can analyze the intensity or time decay of luminescence. There are numbers of publications devoted to the family of optical chemical sensors [60].

Applications include new instrumental methods for clinical chemistry, analytical chemistry, medical diagnosis and therapy, in vivo monitoring of metabolites and nutrients, product sensing during fermentation, remote sensing in dangerous environments.

6.2. Biosensors. The biosensors may be constructed from silicate sol-gels with encapsulated of enzymes and proteins. These systems can detect biologically relevant analytes like O_2 , CO, NO, glucose and oxalate. For biorecognition of specific compounds, enzymes and metalloproteins may be used. The recognition of dissolved oxygen is possible with use of hemoglobin and myoglobin. Myoglobin can also bind CO, and sol-gel with entrapped myoglobin can be used as the sensor for CO by taking advantages of the changes in the absorption spectrum due to protein(CO interaction [61]. The biosensor based on manganese myoglobin is able to detect nitric oxide. Glucose biosensor based on sol-gel with entrapped glucose ox-

idase in combination with horseradish peroxidase and dye precursors (4-aminoantipyrine and p-hydroxybenzene sulfonate), was developed by the same group.

Silica sol-gel encapsulation of the lactate dehydrogenase and its cofactor was employed as a disposable sensor for L-lactate [62]. Reliable measurements of L-lactate are of great interest in clinical chemistry, the dairy and vine industry, biotechnology, or sport medicine.

Glucose oxidase is by far the most studied enzyme for applications in the field of medicine and food technology. Immobilized in silica sol-gel matrix on molecular sieve may be used to prepare the construction for a flow-injection analytical system based on chemiluminescence of luminol• $K_3Fe(CN)_6 \cdot H_2O_2$ to determine glucose concentration [63]. It is also possible to make implantable glucose sensor for in vivo measurements [64,65].

6.3. Metal sensors and pH sensors. Various agents sensitive to selected analytes may be immobilized in monolithic (glassy) xerogel blocks. By monitoring of the characteristic color changes in the doped materials, it is possible to detect the metal cations (Fe^{2+} , Al^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+}), the inorganic anions e.g. SO_4^{2-} , the organic analytes (e.g. orthophenanthroline), pH changes [66]. The sol-gel encapsulated aequorin (the bioluminescent protein found in the jellyfish *Aequorea aequorea*) offers possibility for the development of an optical biosensor for the determination of calcium ions [67].

Doped sol-gel films may offer a simple, non-invasive, reusable and fast optical chemical sensing technique [68]. For example, the TMOS based sol-gel films doped with bromophenol blue and eriochrome cyanine RC served for measuring pH (from 3 to 8) and detection of Cu^{2+} in solutions. The sol-gel entrapment of xylitol orange (XO) provided optical sensing films selective and sensitive to bismuth(III), with low leaching characteristics, fast response, and long life time [69]. Detection of heavy metals with porphyrin sol-gel sensor is possible, as well [70]. A dual-transducer approach based on sol-gel optical sensors was proposed to measure acid and salt concentrations in concentrated aqueous and HCl solutions [71,72]. Various other constructions of sol-gel based pH sensors are described [73–81].

The pH optical fiber sensor without any pH-sensitive dye was also described [82]. Porous silica layer made by the sol-gel method was cladded onto optical fibre core and was exploited as the optical transducer. Acid-base properties of silica surface caused that the surface charge of silica changed with pH of the solution.

6.4. Pesticides, herbicides, and insecticides sensors. There is a constant demand for systems monitoring the water quality and detecting organophosphates. These neurotoxic compounds irreversibly inhibit the enzyme acetylcholinesterase, which is essential for functioning of the central nervous system of mammals and insects. Various solutions were proposed. The sol-gel coated infrared optical sensors enable reproducible detection of organophosphates down to the sub-ppm concentration range [83]. The design and implementation of a portable fiber-

optic cholinesterase biosensor for the detection and determination of pesticides carbaryl and dichlorvos was built as a three-layer sandwich [84]. The enzyme cholinesterase was immobilized on the outer layer, consisting of hydrophilic modified polyvinylidene fluoride membrane and an intermediate sol-gel layer that incorporated bromocresol purple, deposited on an inner disk. Molecularly imprinting sol-gel using a sacrificial spacer was successful in creating a material that could bind DDT [85] with a moderate degree of selectivity.

6.5. Gas sensors. Sol-gel porous materials may be exploited for construction of gas sensors. The specially designed porous networks of SnO₂ nanoparticles for an optical detection of gaseous CO was proposed [86]. The detection of chemical species (toxic gases) by using TiO₂ porous films suitably functionalized with metal-phthalocyanine macromolecules (Me-phthalocyanine, where Me=Fe, Pd or Cu) has been successfully carried out [87]. The Japanese scientists tested the fiber optic carbon dioxide sensor [88], prepared by dip-coating method – the sol-gel film containing indicator dye, thymol blue, was deposited on unclad fiber.

The miniature fiber optic NO₂ sensors based upon dye encapsulation in the silica sol-gel can produce linear and reproducible results [89]. The nitrogen dioxide sensor based on immobilization of ruthenium complex [Ru(bpy)₃]Cl₂ demonstrated sensitivity in the hundreds parts per million range with reversibility and rapid response time. It was also shown that it is possible to build a colorimetric detector of the chemical warfare agent – mustard gas (HD, 1,1-thiobis(2-chloroethane)) [90]. Optical sensors for the measurement of carbon dioxide in modified atmosphere packaging (MAP) applications were also developed [91,92].

Optical sensors for oxygen measurement are attractive since they can be fast, do not consume oxygen and are not easily poisoned. Porous sol-gels may be a base for development of a fiber-optic oxygen sensor. The most common method adopted in construction is based on quenching of fluorescence from appropriate chemical species [93]: Various solutions are already proposed. A thin film dissolved oxygen sensor was fabricated by entrapping erythrosin B in a sol-gel matrix [94]. The first example of the oxygen sensor based on ruthenium(II) polypyridyl dye covalently incorporated onto a silica glass film was described [95]. The O₂-sensing materials based on spin-coated n-octyl-triethoxysilane (Octyl-triEOS) / tetraethylorthosilane (TEOS) composite xerogel films was synthesized and investigated [96]. Porphyrin-doped silica sol-gel glass was used as a probe for gaseous oxygen sensing [97]. In our group we worked on ruthenium based sensors [98].

Hydrogen sensing is also possible. The line-type sensor that can detect the hydrogen leak along the optical fiber was reported [99]. It was demonstrated that thionine dye entrapped in methyltrimethoxysilane (MTMS) film matrix may be used for hydrogen sulfide gas sensing [100].

6.6. Other sensors. Various sol-gels entrapped compounds may be exploited for construction of plethora of sensors. Nano-size particles in MgO films prepared by the sol-gel tech-

nique were used for humidity sensor [101]. The members of Wolfbeis team constructed an optical sensor for ammonia [102,103].

Construction of the optode for urea biosensor requires immobilisation of protein (and pH indicator) in the host matrix. There are several methods enabling protein entrapment. One can use gels, polymers, saccharose, various meshes and membranes [104]. The way of enzyme entrapment has been described proposing the application of sol-gel matrices [105,106].

The physical basis for the design of an optical fiber sensor suited for aqueous medium and gas phase based on the excitation of an evanescent wave at the core/cladding interface was presented [107]. The quantitative toluene detection was based on the refractive index changes (between 1.41 and 1.45) of the silica sol-gel polymer, deposited on the unclad part of the PCS fiber with core diameter 400 μm.

Diffusion of different amines led to changes in the spectra of the silica sol-gel matrix containing the Co(III)porphyrin [108]. This phenomenon was base of the novel amines sensor production.

6.7. Sensor arrays. The combination of pin printing and sol-gel processing techniques provides a simple method to prepare a reusable chemical sensor element. This methodology also provides a straightforward means to fabricate reusable sensor arrays for simultaneous multianalyte quantification.

A new approach to rapidly produce micrometer-scale sensor elements into reusable multianalyte chemical sensor array was already described [109]. By using pin printing technology and sol-gel processing methods, the discrete silica xerogel-based microsensors on a planar substrate (glass microscope slides) were formed. Simultaneous measurement of oxygen concentration and pH was possible by forming discrete O₂ and pH-responsive sensing elements into arrays that allowed to determine O₂ and pH in aqueous solutions.

Integrated optical array sensor platform for oxygen sensing was demonstrated [110]. This sensor scheme was in form of array of 100 discrete O₂-responsive sensing elements on the face of a single LED. The LED served as the light source to excite chemically responsive luminophores (ruthenium(II) complex) sequestered within the doped silica xerogel microsensors and the analyte-dependent emission from the doped xerogel was detected with a charge couple device (CCD).

6.8. Biorecognition, bioimprinting. Quantitative and qualitative analytical techniques are of considerable importance in various aspects of life sciences for the purpose of detection, identification and measurement of concentrations of biologically important molecules, cells, chemical compounds, etc. Molecularly imprinted polymers (MIPs) are of growing interest for their potential biotechnological applications. Recently, the templating processes with living yeast cells were reported [111] for the preparation of ordered and porous silica and titanium oxide sol-gels. A versatile procedure to form regular patterns of molded polymer and sol-gel surfaces with yeast as the templates, which results in packed receptor sites directly

on the coated transducer surfaces, was described. Compared to polymer sensor coatings, the sol-gel layers appeared more robust.

The group of scientists from Singapore proposed a fiber optic biosensor for monitor mutants streptococci activity in human saliva [112]. The developed biosensor was based on the selective bacterial growth medium and evanescent wave spectroscopy at the core-cladding interface of a multimode fiber to monitor the bacterial mediated biochemical reaction. During experiments, the mutants streptococci mediated reaction with sucrose was monitored using a photosensitive indicator bromophenol blue, which was immobilized within the porous silica glass coating. The similar optical set-up was used for monitoring of the bacterial activity by the means of the evanescent wave spectroscopy [113].

The same scientific group developed above mentioned technique and presented a novel method as well as instrumental system for determination of the total protein concentration in a liquid samples [114]. Their fiber optic total protein sensor (FOPS) applied a dye-immobilized (highly sensitive Coomassie Brilliant Blue) porous glass coating on a multi-mode optical fiber. The evanescent waves at the fiber optic core-cladding interface were used for monitoring of the protein-induced changes in the sensor element. The evanescent waves from the optical fibers excited and sensed the presence of protein (presented the calibration curves of bovine serum albumin, haemoglobin, cytochrome c, and ovalbumin) via colour changes in the dye-doped silica sol-gel film along the cladding denuded section.

Genetically modified bacteria, engineered to generate a quantifiable signal in response to pre-determined sets of environmental conditions, may serve as combined sensing/reporting elements in whole-cell biosensors. Joint project of scientist from Germany and Israel [115] was dedicated to compare of two of the several available reporter genes in such cells: green fluorescent proteins (GFPs) (*Aquorea victoria gfp*) and bioluminescence (*Vibrio fischeri luxCDABE*) genes, fused to either SOS (*recA*) or heat shock (*grpE*) promoters. In both cases, bacterial bioluminescence allowed faster and more sensitive detection of the model toxicants; the fluorescent reporter proteins were more stable, and following long-term exposure allowed detection at levels similar to that of the bioluminescent sensors.

The development of fiber-optic biosensors requires that a biorecognition element and a fluorescent reporter group be immobilized at the surface or near the surface of an optical element such as planar waveguide or optical fiber. A model biorecognition element-reporter group couple, consisting of human serum albumin (HSA) that was site-selectively labeled at Cys-34 (single free cysteine) with iodoacetoxynitrobenzoxadiazole (NBD), was entrapped into a variety of different sol-gel derived structures [116]. HSA-NBD was immobilized onto the distal end of the fused silica optical fiber *via* entrapment into sol-gel processed beads or thin films, resulting in a prototype of a reagentless fiber-optic biosensor. The entrapped protein was excited using He-Cd laser.

7. Closing remarks

The review clearly demonstrates that porous materials prepared by sol-gel route possess many interesting features that may be exploited in various practical applications. Silica based materials have potential to be implemented in optical devices, like optical sensors and active coatings. From the other hand, this procedure allows to produce metal doped protective coatings and bioactive compounds for incorporation into prostheses or implants. This paper reports mainly on bioapplications, however, many other possibilities exist where porous sol-gels may have practical significance.

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