Design of nanostructured siloxane-gelatin coatings: Immobilization strategies and dissolution properties

M. Martinez-Ibañez[a,*], I. Aldalur[a], F.J. Romero-Gavilán[b], J. Suay[b], I. Goñi[a], M. Gurruchaga[a]

[a] *Facultad de Ciencias Químicas, Universidad del País Vasco, P. M. de Lardizábal, 3, San Sebastián 20018, Spain*
[b] *Departamento de Ingeniería de Sistemas Industriales y Diseño, Universitat Jaume I, Av. Vicent Sos Baynat s/n, Castellón 12071, Spain*

A B S T R A C T

Owing to the outstanding service life of metallic prostheses, a substantial effort has been put into their surface modification to improve biocompatibility and reduce metallic ion diffusion. To satisfy these requirements, the coating materials obtained using the sol-gel method, with its wide range of tuning properties, have been extensively explored. The well-known biocompatibility of these materials makes them good candidates for different biomedical applications. We designed a series of siloxane-gelatin hybrids to be used as coatings for metallic implants or in controlled delivery systems. Two different matrixes were designed based on methyltrimethoxysilane (MTMOS), tetraethoxysilane (TEOS) and 3-glycidoxypropyltrimethoxysilane (GPTMS) alkoxysilane precursors. In one hybrid coating gelatin was physically entrapped and in the other it was linked to the siloxane network by covalent bonds. Synthesis parameters were established by studying the sol-gel reaction using ^{29}Si nuclear magnetic resonance (^{29}Si NMR), which also allowed quantification of the network connectivity. Dissolution and degradation studies showed the effectiveness of GPTMS as a covalent coupling agent between the silica and gelatin phases; it increased the stability of the coatings in aqueous media. The aim of this study was to design a set of hybrid materials with highly tailorable properties, suitable for their potential biomedical applications.

1. Introduction

Metals like stainless steel, cobalt-chromium-based alloys, titanium or titanium alloys are the materials most widely used in the production of biomedical devices for the repair or replacement of hard tissue and load-bearing bone. Approximately 70% of the structural materials used in implants (artificial joints, bone plates, bone screws, and artificial tooth roots) are metallic. These metals have outstanding mechanical properties and long service life; they are resistant to corrosion and are biocompatible [1]. However, they are quite inert and lack bio-functionality (such as bone conductivity) and bioactivity [2]. Moreover, some studies have demonstrated that metallic ions can diffuse from the implant surface to surrounding tissues, with detrimental effects [3–5]. Hence, a substantial effort has been put into the design of surface modification of metal-based biomaterials. Bioactive ceramics or polymers have been used to develop bio-functional implants and coatings, avoiding the release of harmful metallic particles.

Calcium phosphate coatings have been used to obtain active surfaces because their chemical content resembles the mineral composition of the bone. Metal implants have been coated with inorganic compounds of this type. These compounds are mainly composed of hydroxyapatite (HA), which has bioactive and osteoinductive properties. The osseointegration of such coated implants is faster than of uncoated metal devices [6]; however, the weak adhesion between the HA layer and the metal surface constitutes a critical limitation [7,8]. Some clinical problems have been associated with the failure of adhesion [9].

The demand for alternative methodologies for coating metallic implants has created a widespread interest in the use of sol-gel synthesis route due to their numerous advantages over other coating methods, such as low energy consumption and corrosion protection [10,11]. The silicon alkoxides have been most thoroughly studied among the available metalloid alkoxide precursors. They are widely used in the development of sol-gel biomaterials, mostly due to their recognized biocompatibility and osteoregenerative properties. Therefore, sol-gel route has been widely used in the field of biomaterials for the obtaining of hybrid materials that give rise to monoliths, fibers, powders or films with a wide variety of characteristics [12]. Interestingly, this method allows the deposition of homogeneous coatings on substrates of large and complex curved shapes, by means of various coating methods such as dip-coating, flow-coating or spray coating [13]. Moreover, during

drying and curing step of the sol-gel process, strong covalent metallo-siloxane bonds are created, resulting in excellent adhesion between sol-gel coatings and metal surfaces [14].

TEOS is one of the precursors most often used in the synthesis of bioactive inorganic sol-gel materials [15]. There is also a growing interest in the use of organically modified silicates to obtain hybrid materials with improved properties. Beganskiene et al. [16] have prepared and characterized modified sol-gel derived silica coatings. They have introduced the amino and methyl groups onto the colloidal silica using (3-aminopropyl) triethoxysilane (APTES) and MTMOS, respectively. They have found that the methyl-modified coating is the best substrate supporting cell proliferation. Juan-Diaz et al. [17] have developed functionalized sol-gel coatings by mixing MTMOS and GPTMS (which possesses an epoxide ring) in different molar ratios to improve the osseointegration of titanium implants. The hybrid made using a 1:1 MTMOS:GPTMS ratio showed the best proliferation and mineralization rate. Similarly, hybrids based on MTMOS and TEOS alkoxysilanes improve the mesenchymal stem cells differentiation; this effect increases with the increasing content of the TEOS precursor [18].

The current trends in biomaterials focus on the incorporation of different biomolecules into synthetic polymeric matrixes to mimic the native extracellular matrix (ECM) and regulate the cell behavior. Collagen is the main constituent of native ECM; this protein has been widely used in the pharmaceutical and medical fields. Nevertheless, collagen-containing materials usually suffer from poor physicochemical properties and also have some drawbacks from the material processing point of view [19]. Thus, gelatin replaced collagen in many applications. Most of the recent studies have chosen gelatin because of its biodegradability and bioadhesive properties. Mahony et al. have designed [20] some silica-gelatin scaffolds; these scaffolds have various applications in both hard and soft tissues as their properties can be individually tailored. Lim et al. [21] have incorporated gelatin into a modified polyvinyl alcohol (PVA) hydrogel. This addition does not alter the physical and mechanical properties of the polymer, but significantly improves the cellular interactions. Similarly, Shi et al. [22] have found that gelatin, when grafted onto polycarbonate urethane scaffolds, increases the cell growth and proliferation. The immobilization of such biomolecules can be achieved using: (i) entrapment in the polymeric matrix or (ii) covalent attachment to a polymer carrying pendant reactive groups.

In previous papers [23,24], we reported the benefits of some gelatin/sol-gel coatings on the osteogenesis capability of coated dental implants and the importance of the chemical composition and structure of the material on the release of Si and gelatin. With the aim to go in depth through the knowledge of the synthesis possibilities on the obtaining of formulations with Si and gelatin release modulation capacity, here, we report the synthesis of different gelatin-siloxane materials. Those materials were created using mixtures of three alkoxysilane precursors: MTMOS-TEOS coatings, with various amounts of physically entrapped gelatin, and MTMOS-GPMTS coatings with chemically anchored gelatin in various concentrations. Our objective was to examine the effect of incorporated gelatin on the characteristics of the siloxane network, such as the chemical structure and dissolution rate, focused on the potential applications as coatings for metallic biomaterials or as delivery matrixes.

2. Materials and methods

2.1. Sol-gel films

Organic-inorganic hybrid coatings were synthesized from MTMOS, GPTMS, TEOS and gelatin from porcine skin, Type A. All these reagents were purchased from Sigma-Aldrich and used without further purification. Based on previous studies described in full elsewhere [17,23], the molar ratio between the alkoxysilane precursors MTMOS and TEOS was 7:3 and the ratio for the matrix of MTMOS and GPTMS was 5:5. The

Table 1
Chemical composition of siloxane-gelatin coatings expressed as molar percentage of alkoxysilane precursors and gelatin weight percentage.

Coating	MTMOS (%)	GPTMS (%)	TEOS (%)	Gelatin (wt%)
7M3T	70	–	30	–
7M3T-0.2	70	–	30	0.2
7M3T-0.9	70	–	30	0.9
7M3T-2.0	70	–	30	2.0
5M5G	50	50	–	–
5M5G-0.2	50	50	–	0.2
5M5G-0.9	50	50	–	0.9
5M5G-2.0	50	50	–	2.0

concentration of gelatin in the silica sol was varied (0, 0.2, 0.9 and 2.0 wt%), giving rise to 8 different coatings (Table 1) named 7M3T, 5M5G, 7M3T-0.2, 5M5G-0.2, 7M3T-0.9, 5M5G-0.9, 7M3T-2.0 and 5M5G-2.0.

To obtain 7M3T and 5M5G coatings, the alkoxysilanes were mixed at the appropriate molar ratio, using 2-propanol (Sigma-Aldrich) as solvent (the volume ratio of alcohol to precursor was 1:1). A stoichiometric amount of hydrolyzing solution, an aqueous solution of hydrochloric acid (HCl, pH 1), was added drop-wise as the catalyst of the reaction. The solution was stirred for 2 h to achieve the sol-gel polymerization. For hybrids with gelatin, a mixture of isopropanol and water (1:1 v/v) was used as a solvent to ensure a good miscibility to obtain a homogeneous sol.

The sols were applied onto different substrates. To obtain free films, 3 mL of each sol was poured into a non-stick Teflon mold. The films were used for the chemical characterization of each material. To determine the hydrophilicity of the surface and for the dissolution study, the stainless steel AISI 316-L plates (5 × 5 cm, RNSinox, S.L.) were used as substrate. The plates were polished and cleaned with acetone to remove impurities. After cleaning, the plates were immersed in the sol using a dip-coater (KSV instrument KSV DC) at the speed of 60 mm min^{-1}, left for 1 min, and removed at 100 mm min^{-1}. Then, the plates were heated to obtain a condensed and solid surface (80 °C and 100 °C for 2 h, for 7M3T and 5M5G series hybrids, respectively).

2.2. Chemical characterization

The sol-gel reaction was studied using the liquid state ^{29}Si NMR. The technique was employed to follow the hydrolysis and condensation reaction of the siloxane network. The spectra were recorded on a FT-NMR Bruker 400 Avance spectrometer, at 79.47 MHz. The pulse length was 10 ms, acquisition time 2 s, and the number of scans 384.

Chromium acetylacetonate, Cr(acac)$_3$ was added to the reaction mix at a concentration of 2.5×10^{-3} M as a spin relaxation agent [25]. The external lock solvent was methanol-d_4, and the spectra were referenced to tetramethyl silane. The reaction mixture was prepared, and spectra were recorded before and after adding the catalyst, for up to 2 h.

The solid-state ^{29}Si NMR spectroscopy was used to determine the final structure of the siloxane network and the Si-O-Si cross-linking density achieved after the curing of the films. Cross Polarization Magic Angle Spinning (CP-MAS) NMR spectra were recorded on a 9.4 T Bruker 400 MHZ Avance III WB PLUS spectrometer at 79.5 MHz. Powdered samples were placed in 4-mm rotor tubes. For the spectra acquisition, we used the sample spinning speed of 10 kHz, spectral width of 30 kHz, contact time of 2 ms, and delay time of 5 ms. Peak fitting was employed to determine the relative abundance of each type of silicon atom, T and Q species. Using these data, the network connectivity was quantified and expressed as a degree of condensation (D_C) (Eq. (1)):

$$D_C = \frac{4Q^4 + 3Q^3 + 2Q^2}{4} + \frac{3T^3 + 2T^2}{3} \tag{1}$$

where Q^n represents the abundance of a silicon atom bound to n