Carbohydrate Polymers 93 (2013) 285–290

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Chitosan–silane sol–gel hybrid thin films with controllable layer thickness and morphology

Stefan Spirk[a,b,*,1], Gerald Findenig[a,1], Ales Doliska[b,1], Victoria E. Reichel[a,b], Nicole L. Swanson[a], Rupert Kargl[a,1], Volker Ribitsch[a,1], Karin Stana-Kleinschek[b,1]

[a] Karl-Franzens-Universität Graz, Institut für Chemie, Heinrichstrasse 28/III, 8010 Graz, Austria
[b] University of Maribor, Faculty of Mechanical Engineering, Laboratory for Characterization and Processing of Polymers, Smetanova 17, 2000 Maribor, Slovenia

ARTICLE INFO

Article history:
Received 20 September 2011
Received in revised form 3 April 2012
Accepted 11 April 2012
Available online 27 April 2012

Keywords:
Hybrid materials
Chitosan
Sol–gel process
Thin films
Silanes
Sarfus

ABSTRACT

The preparation of thin films of chitosan–silane hybrid materials by combining sol–gel processing and spin coating is reported. A variety of silanes can be used as starting materials for the preparation of such thin films, namely tetraethoxysilane, tri-*tert*-butoxysilanol, trimethylethoxysilane, *p*-trifluoromethyltetra-fluorophenyltriethoxysilane, trivinylmethoxysilane, (methoxymethyl)trimethylsilane, and hexamethoxydisilane. These silanes are subjected to a sol–gel process before they are added to acidic chitosan solutions. The chitosan:silane ratio is kept constant at 6:1 (w/w) and dilutions with ethanol are prepared and spin coated. Depending on the degree of dilution, film thickness can be controlled in a range between 5 and 70 nm. For the determination of additional surface properties, static water contact angle measurements and atomic force microscopy have been employed.

1. Introduction

Hybrid materials (HM) are able to combine the advantages of organic polymers (e.g. photoluminescence) with those of inorganic compounds, e.g. enhanced stability (Gómez-Romero & Sanchez, 2004; Kickelbick, 2006). The most convenient way to synthesize such HM is based on sol–gel chemistry. This approach enables a simple control of the materials' properties by varying easily accessible experimental parameters including temperature, pH, etc. (Avnir, Klein, Levy, Schubert, & Wojcik, 2001; Corriu & Boury, 2001). Sol–gel processes are widely used to combine synthetic polymers with inorganic parts. In recent years, several approaches for the preparation of hybrid materials using polysaccharides as matrix for inorganic compounds have been described (Chen, Liu, Zhang, & Zhang, 2007; Jun et al., 2010; Rangelova et al., 2008; Samuneva et al., 2008; Schaub, Wenz, Wegner, Stein, & Klemm, 1993; Shchipunov & Karpenko, 2004; Shchipunov, Karpenko, & Krekoten, 2005; Shchipunov, Kojima, & Imae, 2005; Shirosaki et al., 2009; Smitha, Shajesh, Mukundan, & Warrier, 2008).

One major problem of performing sol–gel processes with polysaccharides is the compatibility in terms of solubility, pH-value, hydrophilicity and charge between the sol–gel system and the polysaccharide of interest. If any of the compatibility parameters is not fulfilled, homogenous materials usually cannot be obtained as partial phase separation takes place. Following the criteria of compatibility, some polysaccharides can be incorporated into sol–gel processes easier than others. Among all naturally occurring polysaccharides, chitosan is one of the few which is soluble in aqueous acidic medium due to protonation of the primary amine functionalities. This property makes this poly-β-1-4 glucosamine an interesting candidate for the preparation of HM. Moreover, chitosan features biocompatibility, biodegradability, low toxicity, low cost, enhancement of wound-healing and antibacterial effects; properties which are of great importance in respect to the development of new materials in medical applications (Pavinatto, Caseli, & Oliveira, 2010).

To the best of our knowledge, all chitosan–silane hybrid materials prepared up to now have been synthesized either by reaction of a reactive silane species with the amine functionality (e.g. isocyanate or epoxy substituted alkoxysilanes; Liu, Su, & Lai, 2004; Shirosaki et al., 2009; Silva et al., 2005) thus forming a covalent bond or by crosslinking at the OH groups with hydroxy rich silane compounds such as oligomeric $Si(OH)_4$ yielding a mixture of covalently and hydrogen bonded hybrid materials (Chen et al., 2007; Enescu et al., 2009; Fuentes, Retuert, Ubilla, Fernandez, &

---

* Corresponding author at: Karl-Franzens-Universität Graz, Institut für Chemie, Heinrichstrasse 28/III, 8010 Graz, Austria.
E-mail address: stefan.spirk@uni-graz.at (S. Spirk).

[1] Members of the European Polysaccharide Network of Excellence.

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.04.030

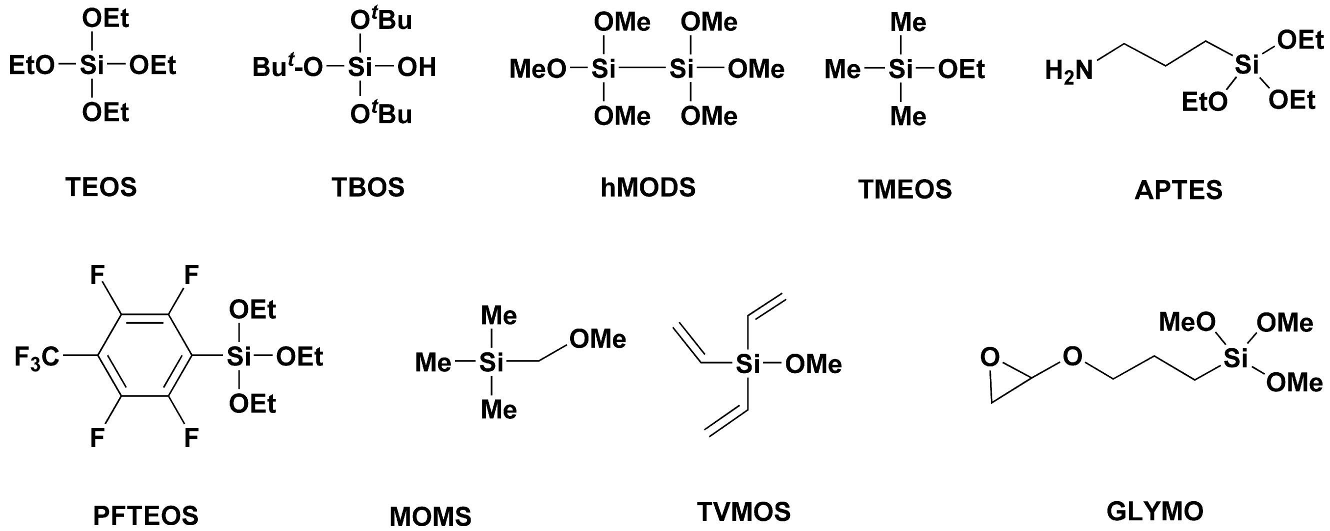

Fig. 1. Silane precursors used for thin hybrid film preparation. TEOS: tetraethoxysilane, TBOS: tri-*tert*-butylsilanol, hMODS: hexamethoxydisilane, TMEOS: trimethylethoxysilane, APTES: aminopropyltriethoxysilane, PFTEOS: 2,3,5,6-tetrafluoro-4-trifluoromethyl-triethoxysilane, MOMS: (methoxymethyl)trimethylsilane, TVMOS: trivinylmethoxysilane, GLYMO: ω-glycidylpropoxytrimethoxysilane.

Gonzalez, 2000; Uragami et al., 2004). The thickness of the resulting films is in the μm to mm range. Several very interesting applications for these materials, e.g. as membranes (Uragami et al., 2004), for tissues (Shirosaki et al., 2009), as scaffolds (Braga et al., 2009; Molvinger, Quignard, Brunel, Boissiere, & Devoisselle, 2004; Shirosaki et al., 2009; Witoon, Chareonpanich, & Limtrakul, 2009; Yan et al., 2009; Yeh, Chen, & Huang, 2007), for better cell proliferation (Shchipunov & Karpenko, 2004), and for enhanced nerve regeneration (Amado et al., 2008) have been described. For a deeper understanding of structure–property relationships in such materials, it would be desirable to produce materials with defined surface morphology and wettability. Therefore, the fabrication of model films is of significant interest (Mohan et al., 2011). The demands on these films vary depending on the intended application. Besides chemical, morphological equivalence is the most important point because surface interactions such as adsorption (e.g. of proteins) or wetting strongly depend on the surface morphology and composition (Kulterer et al., 2012). In this paper, an easy, fast and highly reproducible method to prepare chitosan–silane hybrids on the nanometer range (∼5–70 nm thickness) by sol–gel chemistry is demonstrated.

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation: 85%, $M_w \sim 400{,}000$ Da, practical grade, from crab shells) is obtained from Sigma–Aldrich (Steinheim, Germany) and used without further treatment. Alkoxysilanes (reagent grade) are obtained from ABCR (Karlsruhe, Germany) and used without further purification. Acetic acid (reagent grade) and ethanol (reagent grade) are obtained from Roth (Karlsruhe, Germany).

2.2. Preparation of the hybrid thin films

For the synthesis of prehydrolyzed/precondensed sol–gel solutions, 1.0 g of the alkoxysilane is dissolved in 98.5 g EtOH and 0.5 g acidic water (pH = 1.5, adjusted with HCl) is added. These solutions are stirred for two days in a closed vessel, before they are used for hybrid material preparation. Chitosan solutions are prepared by slowly adding chitosan (1.5 g) to 7 M acetic acid (98.5 g) at 60 °C. This solution is stirred overnight at this temperature, cooled to room temperature and filtered prior to use.

For spin-coating experiments, 1.0 g of the silane solution is added to 4.0 g of the chitosan solution and mixed vigorously for 1 min. Then 100 μl of the resulting solution are deposited onto surfs and subjected to spin coating ($a = 2500$ rpm/s, $v = 4000$ rpm, $t = 60$ s). For the dilution experiments, the stock solution is prepared as mentioned above, diluted with the corresponding amount of EtOH and allowed to crosslink for 1 min. For each dilution, two surfs with a total surface of 1 cm^2 each are coated. After spin coating, the resulting materials are dried for one week at room temperature (relative humidity in our labs on average is 50–70%). Alternatively, drying can be performed for 4 h in a drying oven with adjusted humidity (50%) at 40 °C without changing surface morphology and contact angles.

2.3. Layer thickness determination – Sarfus

Optical microscopy (Sarfus) is done on a polarization light microscope from Leica (Wetzlar, Germany). Surfs are obtained from Nano Lane (Montfort-le-Gesnois, France) and analyzed using the Sarfusoft program. A more detailed description of this method can be found elsewhere (Ausserre & Valignat, 2006). For layer thickness determination, two surfs of each dilution are used. The films are scratched using a stainless steel cannula to obtain a small area which is uncoated. Consequently, the height difference between this uncoated area and the coated part is measured at different positions and height profiles are created using the Sarfusoft software. For layer thickness determination, at least three height profiles have been used on each surf.

2.4. Atomic force microscopy

AFM measurements were performed in tapping mode with an Agilent 5500 AFM multimode scanning probe microscope (Digital Instruments, Santa Barbara, CA). The images were scanned using silicon cantilevers (ATEC-NC-20, Nanosensors, Germany) with a resonance frequency of 210–490 kHz and a force constant of 12–110 N/m. Image processing, analysis and root mean square roughness calculation are performed with WSxM freeware (Horcas et al., 2007).

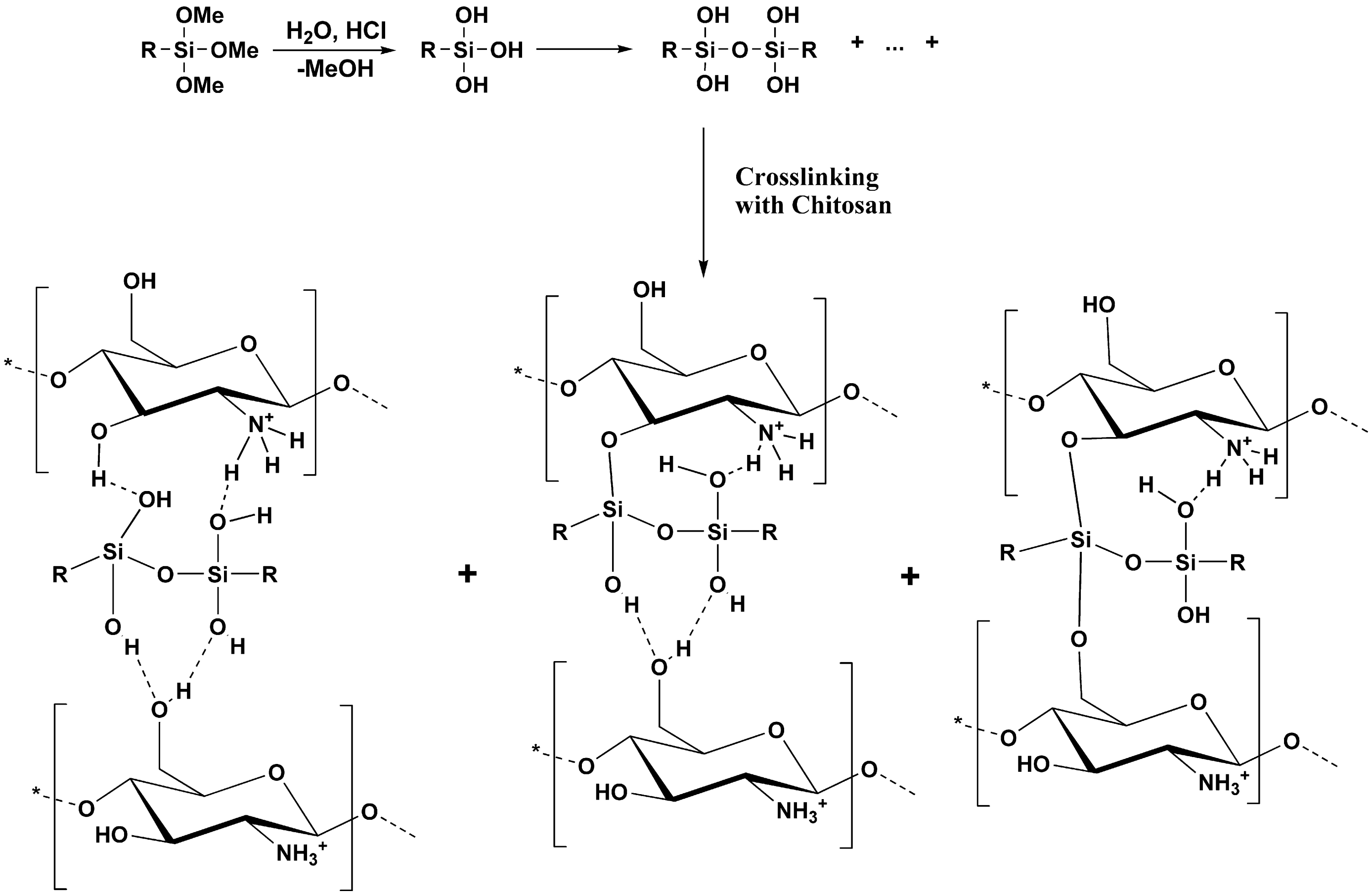

Fig. 2. Schematic representation of the hydrolysis and condensation reactions of trialkoxysilanes followed by crosslinking of their silanol functionalities with OH-functions of chitosan. For clarity reasons, only the interaction of the primary condensation product with one repeating unit of one chitosan molecule is depicted and the acetate counterions are omitted.

2.5. Wettability

Contact angle measurements have been performed on a Dataphysics (Filderstadt, Germany) contact angle system (OCA15+) using water (18 MΩ) as wetting liquid. Drop volume is adjusted to 3 μL. For the determination of the equilibrium structure of the liquid–solid–vapor interface, the sessile drop method has been employed.

3. Results and discussion

From prescreening experiments, we know that film formation properties are superior for materials with high chitosan content. It turned out that in cases where the silane content is too high, the formation of films cannot be observed. Instead, phase separation after spin coating occurs and inhomogeneous films are obtained. Therefore, the chitosan to silane ratio is kept constant at 6:1 (w/w) for all experiments. In a first step, a screening is performed to elucidate which silane precursors are the most suitable ones for thin hybrid film preparation (Fig. 1).

For this purpose, several prehydrolyzed/precondensed alkoxysilanes are added to acidic solutions of chitosan ($M_W \sim 400{,}000$ Da). After mixing, the solutions are allowed to crosslink for 1 min, deposited on Si-wafers and subjected to spin coating. From a thermodynamical point of view, the formation of covalent bonds between the silanes and the chitosan can only proceed via the hydroxyl groups of the chitosan due to the formation of strong Si—O bonds (av. 440 kcal/mol) rather than weak Si—N bonds (av. 320 kcal/mol) (Holleman & Wiberg, 1995). In addition, under the experimental conditions employed for the preparation of the hybrid materials, a potential covalent Si—N bond is unstable and replaced immediately by a silicon—oxygen bond. Calculations in the gas phase (B3LYP/6-311+G*) revealed a reaction free energy (ΔG^{298}) close to 0 kcal/mol for reactions of alkoxysilanes with hydroxyl groups of cellobiose (Spirk, Ehmann, Ribitsch, & Stana-Kleinschek, 2011). Nevertheless, from related cases it is known that activation energy is necessary for the initiation of similar reactions (Spirk, Belaj, Nieger, & Pietschnig, 2009; Spirk, Madl, Belaj, & Pietschnig, 2010). Therefore, the silanes are not only covalently bound to the chitosan but also incorporated into the matrix via hydrogen bonding. Although the amine groups are not forming covalent bonds with the silanes under the employed reaction conditions, they act as excellent hydrogen bonding partners and facilitate the formation of a network and subsequently of a film. Another factor which influences the formation of such a network is the pH which is chosen for materials preparation. Under the acidic conditions employed, the amine group of chitosan is protonated and forms an NH_3^+ ion which in turn is an even better hydrogen bonding partner than an uncharged NH_2 group. A postulated reaction scheme can be found in Fig. 2.

Most of the used silanes form homogenous films with a layer thickness ranging from 65 to 73 nm.

However, a few alkoxysilanes employed in HM preparation are not able to form thin layers on silicon wafers at all. In case of chitosan–*tert*-butyldiphenylmethoxysilane (TBDPMOS) hybrids, foam like structures are obtained. It seems that in the case of

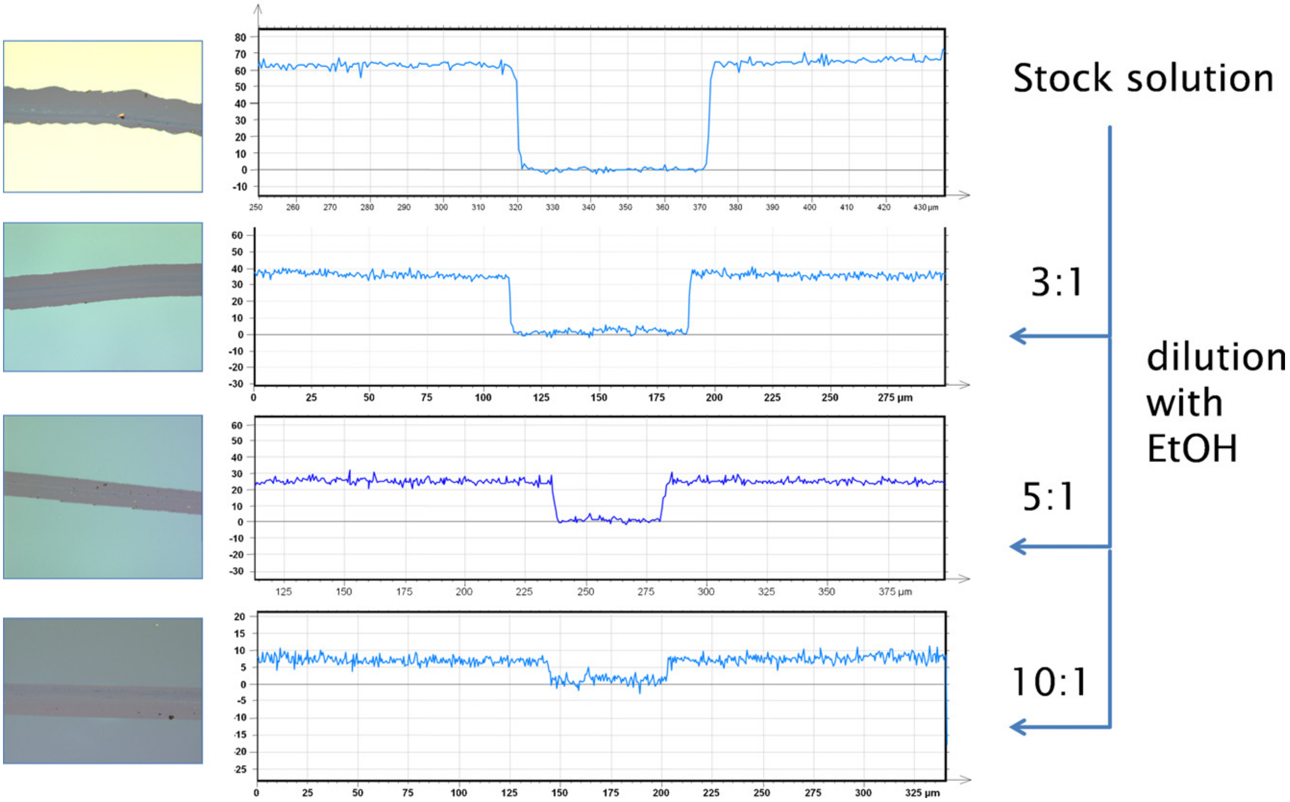

Fig. 3. Left: microscope images (20× magnification, 200 µm × 200 µm) of chitosan–hMODS hybrids prepared by spin-coating. The scratch in the middle of the images has been made using a cannula. Right: height profiles (nm) of these layers determined by Sarfus.

TBDPMOS, the presence of only one hydrolyzable alkoxy group complicates the crosslinking with the chitosan moiety and the incorporation into the chitosan matrix, respectively. Nevertheless, the main reason for the formation of inhomogeneous structures on the surface may originate from the huge steric demand of TBDPMOS which results in a reduced reactivity compared to other monoalkoxysilanes. Another factor which influences the formation of the hybrid materials is the pronounced hydrophobicity of

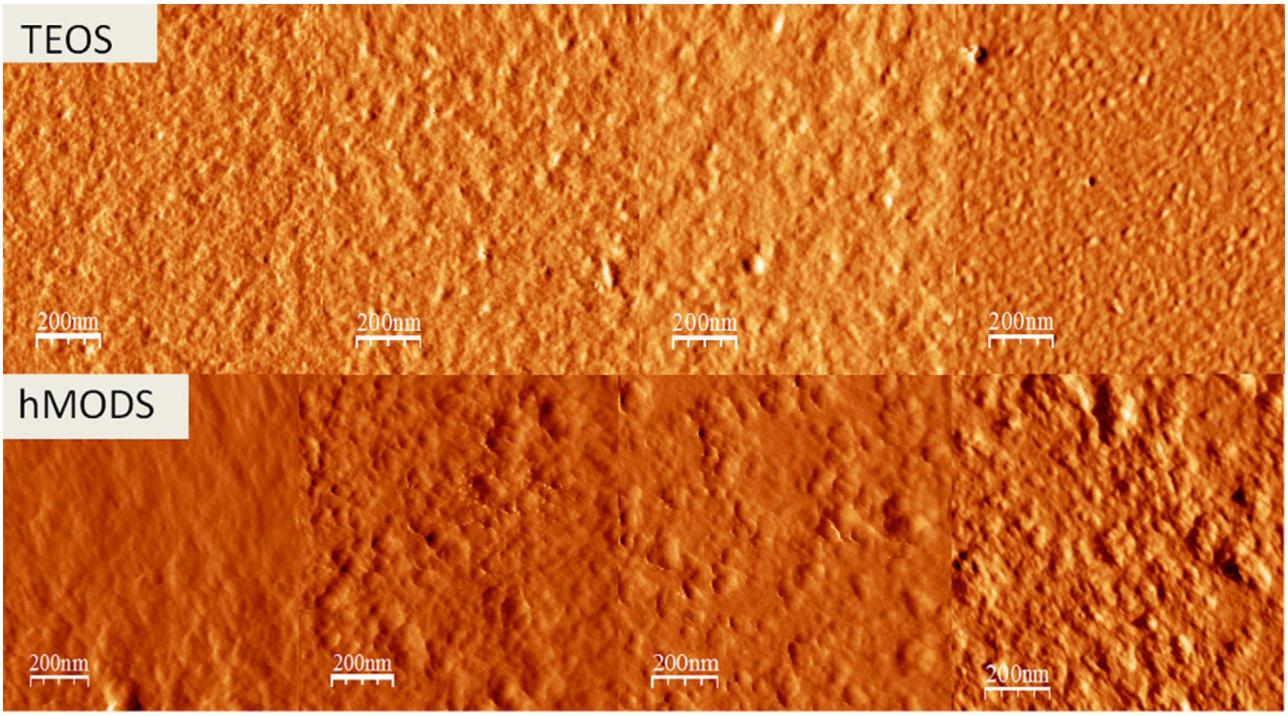

Fig. 4. 1 µm × 1 µm AFM images of selected hybrid thin films (from left to right: undiluted, 3:1, 5:1 and 10:1). Layer thickness decreases from left to right according to Table 1.

Table 1
Summary of the layer thicknesses (nm) of several chitosan silane hybrid films as a function of the degree of dilution. Layer thicknesses have been determined on two different surfs using at least three height profiles on each surf.

	hMODS	MOMS	PFTEOS	TBOS	TEOS	TMEOS	TVMOS
Stock	65(5)	68(2)	73(2)	69(4)	73(2)	66(1)	68(3)
1:1	–	54(3)	55(2)	57(3)	–	58(3)	52(3)
3:1	34(4)	33(3)	27(3)	25(5)	37(1)	29(1)	35(2)
5:1	24(4)	16(2)	22(1)	10(2)	21(1)	16(4)	16(1)
10:1	8(1)	6(1)	6(2)	7(1)	8(2)	7(1)	3(1)

Values in parenthesis represent standard deviations.

the substituents which leads to problems in the incorporation of TBDPMOS into the chitosan matrix and subsequently to phase separation.

The next step is to investigate whether the layer thickness of the HM can be tuned. For this purpose, the solutions are diluted with EtOH in different ratios (1:1, 3:1, 5:1, 10:1) prior to spin-coating. As expected, layer thicknesses decrease with a higher degree of dilution (for a representative example, see Fig. 3). A few silanes like ω-glycidylpropoxytrimethoxysilane (GLYMO) and 3-aminopropyl-triethoxysilane (APTES), form homogenous layers ($d = 65$ and 64 nm) only when using undiluted solutions. Upon dilution, partial phase separation is observed, maybe due to insufficient crosslinking time.

For the highest diluted solutions (10:1) thicknesses vary in a range from 6 (PFTEOS) to 8 nm (TEOS). The only exception is TVMOS where a layer thickness of 3 nm is determined. In case of hMODS and TEOS, homogenous layers cannot be obtained for the smallest dilution (1:1).

A summary of the resulting layer thicknesses for the different silanes and all dilutions is shown in Table 1.

An important issue in model film preparation is how surface morphology is changed with decreasing layer thickness. Ideally, there should not be any major changes of the morphology, however, it is known from, e.g. cellulose model surfaces that especially thin layers (~<10 nm) are very prone to defects thus making reproducible preparation difficult. AFM images of the different hybrid surfaces appear to be very similar regardless of the layer thickness and the chemical composition (Fig. 4).

However, there are minor differences in the roughness of the surfaces and a slight dependence on layer thickness is observed. In these cases, a tendency for particle formation on the surface is pronounced. The only exception is hMODS where thin films (8 nm) differ significantly from thicker ones (65 nm). This particle formation on the surface can be explained by the size of the particles whose diameter is in the range of the layer thickness; therefore for thin films more particles are visible on the surface than for thicker layers.

For further evaluation of the surface behavior, static water contact angles on these surfaces are determined. For the sake of comparison, contact angles are measured immediately after the deposition of the water drops onto the films. For silanes with hydrophobic organic groups like PFTEOS or TMEOS, quite low water contact angles are observed (PFTEOS: 62–72°; TMEOS: 58–67°). Possible explanations for this phenomenon include the short crosslinking time (60 s) before spin coating as well as the low amount of silane present in the material. However, experiments where the crosslinking time has been expanded (1, 2, 4, 8, 12, 24 h) before spin coating did not reveal any significant influence on the contact angles (see supporting information). In contrast, an extension of the drying period after preparation significantly increases the contact angles maybe due to a complete removal of solvents. Moreover, there is also the possibility that the films have to reach a state of equilibrium in respect to their molecular orientation which of course has an impact on the wettability behavior. However, a detailed study on equilibrium studies would exceed the scope and intention of this paper.

Table 2
Static water contact angles (°) on the synthesized thin hybrid films after drying.

	hMODS	MOMS	PFTEOS	TBOS	TEOS	TMEOS	TVMOS
Stock	75(2)	67(2)	72(1)	67(2)	54(2)	58(3)	67(2)
3:1	70(2)	62(2)	62(2)	65(2)	51(2)	67(2)	69(1)
5:1	67(2)	66(3)	64(3)	58(4)	59(2)	63(2)	70(2)
10:1	71(2)	64(1)	66(2)	67(3)	64(1)	65(2)	78(2)

Standard deviations are given in parenthesis.

Any significant dependence of the contact angles on the layer thickness cannot be observed. All other silanes show contact angles between 50° and 80°. A detailed summary of this data can be found in Table 2.

4. Summary and conclusion

A straightforward approach towards a fast and highly reproducible preparation of chitosan–silane hybrid thin films via a combination of sol–gel processing and spin coating is presented. The thickness of the films can be tuned in a range from ~5 to ~70 nm by simple dilution steps. The surface morphology, a key element for surface interactions, is neither influenced by silanes having different chemical structure nor by decreasing layer thicknesses. It has to be mentioned that the molecular size which is somewhat related to the molecular weight of the chitosan influences also the layer thickness of these films. The incorporation of the silanes into the matrix as well as the reactivity of the chitosan are affected to a large extent by the size of the chitosan. Preliminary results using chitosan with lower and higher mass compared to the one used in this study showed layer thicknesses to be between 50 and 90 nm using the same procedure. Therefore the choice of the chitosan allows another possibility for the tuning of the layer thickness of the hybrid materials which will be the topic of an upcoming paper. Future investigations will also focus on how these materials may be incorporated for the studying of surface interactions with proteins and living cells.

Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme [FP7/2007–2013] under grant agreement no. 214015 and 214653. We would also like to thank Dr. Stefan Köstler and Simon Lenz for fruitful discussions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.04.030.

References

Amado, S., Simoes, M. J., Armada da Silva, P. A. S., Luis, A. L., Shirosaki, Y., Lopes, M. A., et al. (2008). Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model. *Biomaterials*, *29*(33), 4409–4419.

Ausserre, D., & Valignat, M.-P. (2006). Wide-field optical imaging of surface nanostructures. *Nano Letters*, *6*(7), 1384–1388.

Avnir, D., Klein, L. C., Levy, D., Schubert, U., & Wojcik, A. B. (2001). Organo-silica sol gel materials. In Z. Rappoport, & Y. Apeloig (Eds.), *Chemistry of organic silicon compounds* (pp. 2317–2360). Chichester, UK: Wiley.

Braga, T. P., Gomes, E. C. C., Sousa, A. F., Carreno, N. L. V., Longhinotti, E., & Valentini, A. (2009). Synthesis of hybrid mesoporous spheres using the chitosan as template. *Journal of Non-Crystalline Solids*, *355*(14–15), 860–866.

Chen, J. H., Liu, Q. L., Zhang, X. H., & Zhang, Q. G. (2007). Pervaporation and characterization of chitosan membranes cross-linked by 3-aminopropyltriethoxysilane. *Journal of Membrane Science*, *292*, 125–132.

Corriu, R. J. P., & Boury, B. (2001). Nanostructured hybrid organic–inorganic solids from molecules to materials. In Z. Rappoport, & Y. Apeloig (Eds.), *Chemistry of organic silicon compounds* (pp. 565–640). Chichester, UK: Wiley.

Enescu, D., Hamciuc, V., Ardeleanu, R., Cristea, M., Ioanid, A., Harabagiu, V., et al. (2009). Polydimethylsiloxane modified chitosan. Part III: Preparation and characterization of hybrid membranes. *Carbohydrate Polymers*, *76*, 268–278.

Fuentes, S., Retuert, P. J., Ubilla, A., Fernandez, J., & Gonzalez, G. (2000). Relationship between composition and structure in chitosan-based hybrid films. *Biomacromolecules*, *1*, 239–243.

Gómez-Romero, P., & Sanchez, C. (2004). *Functional hybrid materials*. Weinheim, Germany: Wiley-VCH.

Holleman, A. F., & Wiberg, N. (1995). *Lehrbuch der anorganischen Chemie*. New York, USA: Walter de Gruyter.

Horcas, I., Fernandez, R., Gomez-Rodriguez, J. M., Colchero, J., Gomez-Herrero, J., & Baro, A. M. (2007). *Review of Scientific Instruments*, *78*, 013705.

Jun, S.-H., Lee, E.-J., Yook, S.-W., Kim, H.-E., Kim, H.-W., & Koh, Y.-H. (2010). A bioactive coating of a silica xerogel/chitosan hybrid on titanium by a room temperature sol–gel process. *Acta Biomaterialia*, *6*, 302–307.

Kickelbick, G. (2006). *Hybrid materials*. Weinheim, Germany: Wiley-VCH.

Kulterer, M. R., Reichel, V. E., Kargl, R., Köstler, S., Sarbova, V., Heinze, T., et al. (2012). Functional polysaccharide composite nanoparticles from cellulose acetate and potential applications. *Advanced Functional Materials*, *22*(8), 1749–1758.

Liu, Y.-L., Su, Y.-H., & Lai, J.-Y. (2004). In situ crosslinking of chitosan and formation of chitosan–silica hybrid membranes with using γ-glycidoxypropyltrimethoxysilane as a crosslinking agent. *Polymer*, *45*, 6831–6837.

Mohan, T., Kargl, R., Doliška, A., Vesel, A., Köstler, S., Ribitsch, V., et al. (2011). Wettability and surface composition of partly and fully regenerated cellulose thin films from trimethylsilyl cellulose. *Journal of Colloid and Interface Science*, *358*, 604.

Molvinger, K., Quignard, F., Brunel, D., Boissiere, M., & Devoisselle, J.-M. (2004). Porous chitosan–silica hybrid microspheres as a potential catalyst. *Chemistry of Materials*, *16*, 3367–3372.

Pavinatto, F. J., Caseli, L., & Oliveira, O. N. (2010). Chitosan nanostructured thin films. *Biomacromolecules*, *11*, 1897.

Rangelova, N., Chernev, G., Nenkova, S., Samuneva, B., Georgieva, N., Yotova, L., et al. (2008). New silica hybrid nanomaterials containing pectin and alginate. *Nanoscience & Nanotechnology*, *8*, 246–249.

Samuneva, B., Djambaski, P., Kashchieva, E., Chernev, G., Kabaivanova, L., Emanuilova, E., et al. (2008). Sol–gel synthesis and structure of silica hybrid biomaterials. *Journal of Non-Crystalline Solids*, *354*, 733–740.

Schaub, M., Wenz, G., Wegner, G., Stein, A., & Klemm, D. (1993). Ultrathin films of cellulose on silicon wafers. *Advanced Materials*, *5*(12), 919–922.

Shchipunov, Y. A., & Karpenko, T. Y. (2004). Hybrid polysaccharide–silica nanocomposites prepared by the sol–gel technique. *Langmuir*, *20*(10), 3882–3887.

Shchipunov, Y. A., Karpenko, T. Y., & Krekoten, A. V. (2005). Hybrid organic–inorganic nanocomposites fabricated with a novel biocompatible precursor using sol–gel processing. *Composite Interfaces*, *11*, 587–607.

Shchipunov, Y. A., Kojima, A., & Imae, T. (2005). Polysaccharides as a template for silicate generated by sol–gel processes. *Journal of Colloid and Interface Science*, *285*, 574–580.

Shirosaki, Y., Tsuru, K., Hayakawa, S., Osaka, A., Lopes, M. A., Santos, J. D., et al. (2009). Physical, chemical and in vitro biological profile of chitosan hybrid membrane as a function of organosiloxane concentration. *Acta Biomaterialia*, *5*(1), 346–355.

Silva, S. S., Ferreira, R. A. S., Fu, L., Carlos, L. D., Mano, J. F., Reis, R. L., et al. (2005). Functional nanostructured chitosan–siloxane hybrids. *Journal of Materials Chemistry*, *15*, 3952–3961.

Smitha, S., Shajesh, P., Mukundan, P., & Warrier, K. G. K. (2008). Sol–gel synthesis of biocompatible silica–chitosan hybrids and hydrophobic coatings. *Journal of Materials Research*, *23*(8), 2053–2060.

Spirk, S., Belaj, F., Nieger, M., & Pietschnig, R. (2009). Formation and hydrogen bonding of a POSS-trisilanol. *Dalton Transactions*, 163.

Spirk, S., Ehmann, H., Ribitsch, V., & Stana-Kleinschek, K. (2011). Silylation of cellobiose as a model reaction for the synthesis of silylated cellulose. A DFT and PM3 approach. In *Proceedings of the 15th int. electron. conf. Synth. Org. Chem.: Sciforum Electronic Conferences Series*

Spirk, S., Madl, T., Belaj, F., & Pietschnig, R. (2010). A radical approach to hydroxylaminotrichlorosilanes: Synthesis, reactivity, and crystal structure of TEMPO-$SiCl_3$ (TEMPO = 2,2,6,6-tetramethylpiperidine-*N*-oxyl). *European Journal of Inorganic Chemistry*, 289–297.

Uragami, T., Katayama, T., Miyata, T., Tamura, H., Shiraiwa, T., & Hikaguchi, A. (2004). Dehydration of an ethanol/water azeotrope by novel organic–inorganic hybrid membranes based on quaternized chitosan and tetraethoxysilane. *Biomacromolecules*, *5*, 1567–1574.

Witoon, T., Chareonpanich, M., & Limtrakul, J. (2009). Effect of acidity on the formation of silica–chitosan hybrid materials and thermal conductive property. *Journal of Sol–Gel Science and Technology*, *51*(2), 146–152.

Yan, E., Ding, Y., Chen, C., Li, R., Hu, Y., & Jiang, X. (2009). Polymer/silica hybrid hollow nanospheres with pH-sensitive drug release in physiological and intracellular environments. *Chemical Communications*, *19*, 2718–2720.

Yeh, J.-T., Chen, C.-L., & Huang, K.-S. (2007). Synthesis and properties of chitosan/SiO_2 hybrid materials. *Material Letters*, *61*(6), 1292–1295.