

# Biotemplating: polysaccharides in materials engineering

C. Zollfrank

*Bioengineered Ceramics, Department of Materials Science and Engineering 3 - Glass and Ceramics, University of Erlangen-Nuremberg, Erlangen, Germany*

## Abstract

The generation of biominerals and hard tissue in nature is directed and templated by biomacromolecules. In many systems, polysaccharides play a significant role during the assembly of the inorganic phase. Polysaccharides exhibit a hierarchical multiscale order as well as self-assembly properties and they appear in a large variety of structures. The directed deposition of inorganic phases on polysaccharide templates is an interesting approach for the manufacturing of patterned functional materials. This paper highlights our recent developments on the bioinspired deposition and formation of inorganic phases on polysaccharide templates. Polysaccharides can be used at various structural levels from the molecular scale to three-dimensional parts in the millimetre range. The versatility of polysaccharide templating capabilities will be shown on one-dimensional cellulose nanocrystals for formation of luminescent inorganic nanotubes for optoelectronic applications. The replication of complex plant tissue and processed lignocellulosic materials such as paper and cardboard into carbide based engineering and functional ceramics will be presented. The developed methods for the mineralisation of inorganic phases on polysaccharides are adapted for an innovative bioinspired route involving phototactic microorganisms, which are useful for the light-induced fabrication of hierarchically structured functional materials.

*Keywords: biotemplating, functional materials, structural ceramics, engineering ceramics.*



# 1 Introduction to biotemplating

The demand for multiscale, nanostructured ceramic materials with a controlled microstructure and tailored properties at an even smaller length scale beyond 100 nm is still growing [1–4]. Most of the applied manufacturing techniques for nano-scaled functional ceramics are top-down strategies limiting the accessibility of nano-scaled microstructures to minimum sizes of around 100 nm. Biological systems are able to produce most complex inorganic structures and morphologies [5–7] from the nano to the macro scale. Biomimetic and bio-inspired growth and shaping of inorganic matter are subject of current research activities [3, 4, 8–10]. Naturally formed biopolymer composites and biominerals [6] attracted considerable attention and advanced materials manufacturing processes combined with nano-biotechnological methods have emerged [11]. In natural systems such as wood polysaccharides are hierarchically organised on the various structural levels ranging from the nano to the macroscopic length scale, figure 1.

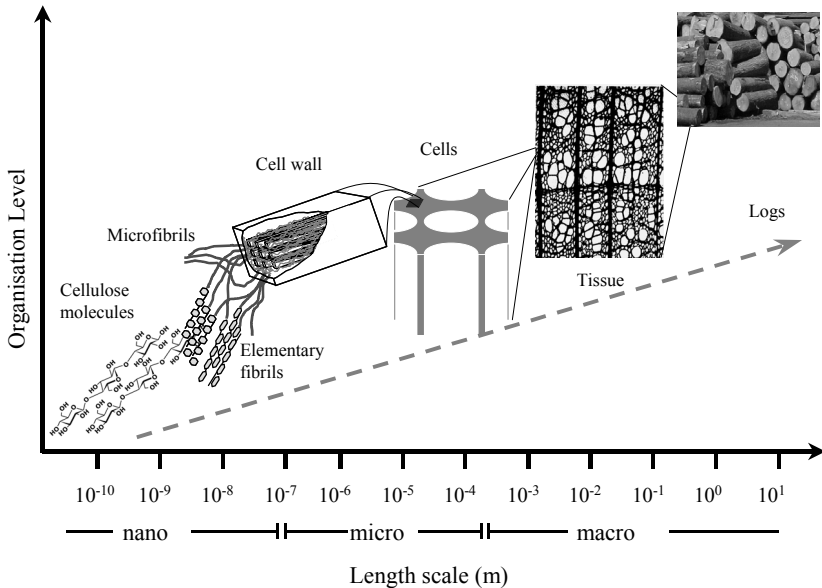


Figure 1: Structural hierarchy of polysaccharides in wood: Organisation level versus length scale.

A current trend in materials design and fabrication is to produce materials with hierarchical structures and multi-components at various scales including hybrid functions [12]. The envisioned multipurpose materials, which exhibit a hierarchical organisation, multifunctions, environmental resistance and stimuli-response including adaptability are readily found in biological materials. Biological materials have been optimised in evolutionary processes. A hierarchical

organisation scaled from nano-, micro- to macro-sizes is a common feature of almost all biological materials [13–15]. They often show complex and unusual structures, which are difficult to artificially reproduce in a cost-effective manner.

The design and fabrication of large supramolecular structures with a hierarchical and complex three-dimensional morphology have been successfully achieved in organic chemistry, whereas the creation of hierarchical inorganic structures is lagging behind [10]. The use of templates is a commonly applied method in materials chemistry to realise novel structures from the macro down to the nanometre scale. Almost any (in)soluble organic material structure might act as template. These are low molecular weight molecules, polymers, supra-molecular assemblies, tissues, cells or even microorganisms. The templates are involved during the materials generation process by providing a precursor structure for the final material form. Templating consequently can be defined as inorganic phase formation by transcription of this precursor structure. The process of templating can be divided into several consecutive steps, figure 2:

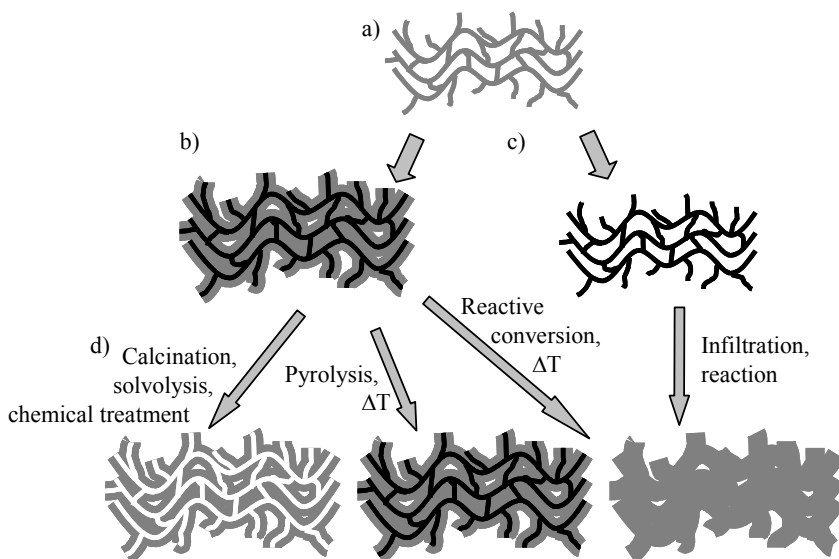


Figure 2: Biotemplating: a) multidimensional organised (bio-)organic template, b) infiltration and/or reaction with inorganic phase to form a hybrid material, c) conversion of the biotemplate retaining the original structure, d) final materials formation processing.

A bioorganic template is brought into contact with an inorganic precursor or small particles of the inorganic material that will be formed. This is usually performed in solution, but can also be carried out in the gas phase. Deposition of the inorganic material on the inner or outer surface of the template will result in the formation of an organic/inorganic hybrid material. Optionally, this organic/inorganic hybrid material might already exhibit interesting potential

applications [8, 11, 12]. The organic template material can be removed through heat treatment, calcination, washing with solvents or other chemical treatments. This results in the final inorganic material with a morphology directly related to the (bio)organic template. Calcination treatment or chemical removal of the templates usually yields porous inorganic structures, whereas a carbon/inorganic composite is accessible through pyrolysis. The hybrid materials composite can be also converted into a dense inorganic material by solid-state reactions at elevated temperatures. In some cases, the template itself has to be converted into a different, more active material preserving the original structure prior to a reactive conversion. For example, a bioorganic structure can be transformed into carbon by pyrolysis, which is subsequently transformed into biomorphous carbide ceramics by reactive melt infiltration [16]. In general, biotemplating refers to the use of natural occurring structured resources as starting materials for the manufacturing of novel inorganic compounds [12, 14, 17–19], which are promising materials for a variety of applications (light-weight materials, ballistic protection, energy systems, filters and catalysts, tribological devices, photocatalysis). In the following chapters the benefits of polysaccharide biotemplates will be demonstrated on selected examples covering several length scales. One major research focus of our work is the multiscale (hierarchical) design of ceramic functional materials using organised biomacromolecules (cellulose nanocrystals), biological templates (wood, algae) as well as processed biological materials (paper, cardboard). This innovative approach has led to a variety of functional ceramic materials with tailored properties [14].

## 2 Biotemplating with cellulose nanocrystals

Cellulose nanocrystals can be fabricated by hydrolysis in concentrated mineral acids, such as sulphuric or hydrochloric acid, from different cellulose sources, such as cotton [20, 21], wood pulp [22], sugar beet pulp [23], marine tunicate cellulose [24] and cellulose of bacterial or algal origin [25]. The hydrolysis time, acid concentration and ultrasonic treatment have a strong influence on particle size and aggregation characteristics and have been extensively studied [26, 27]. The cellulose structures might also react as templates in wet-chemical coating processes such as layer-by-layer deposition. The sol-gel process was used to coat cellulose fibres with photoactive TiO<sub>2</sub> films [28], cellulose nanocrystals with TiO<sub>2</sub> [29] and cellulose nanorod nematic suspensions with SiO<sub>2</sub>, leading to a mesoporous replica upon template removal [30]. We recently reported on the templated formation of silica nanotubes on molecular polysaccharide derivatives by polycondensation of silicic acid [31] and sol-gel processing [32], figure 3.

The cellulose nanocrystals, figure 3a), were coated with silica in an acid-catalysed sol-gel process using tetraethylorthosilicate (TEOS) and acetic acid yielding a cellulose/silica nanocomposite, figure 3b). The cellulose templates were removed by calcination at 650°C, figure 3c). Using these procedures, silica nanotubes with diameters of 15 nm and lengths up to 500 nm can be obtained. The absence of rings in electron diffraction patterns indicated the lack of a

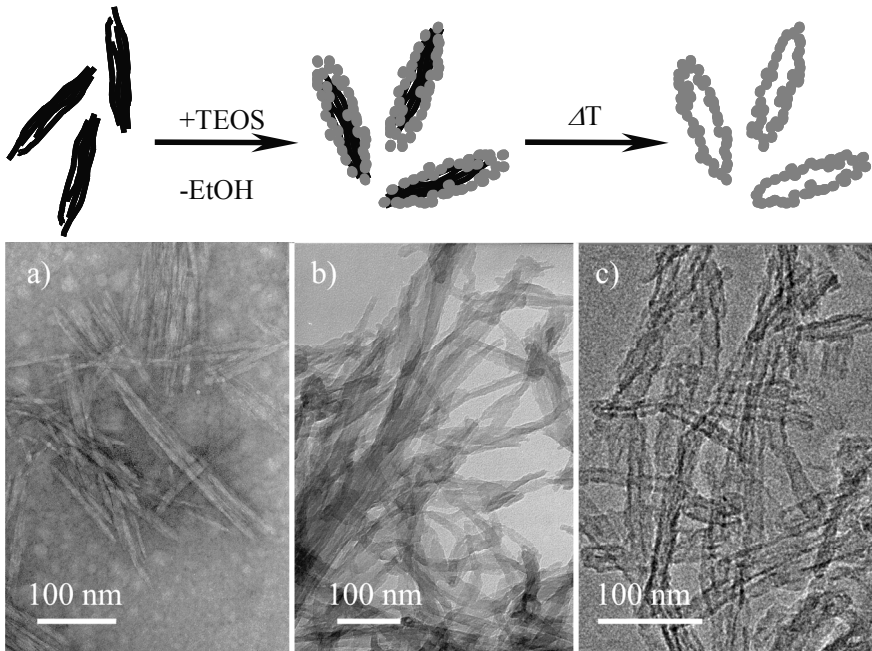


Figure 3: Biotemplating using a sol-gel process: Coating of cellulose nanocrystals with silica from an organosilicon precursor and subsequent calcination: a) cellulose nanocrystals, b) cellulose/silica composites, c) silica nanotubes.

distinct crystallinity of the silica nanotubes. Cathodoluminescence spectra of calcined samples showed a blue emission at 450 nm following irradiation with 150 keV electrons [32].

### 3 Biomorphous carbide ceramics from cardboard

Silicon carbide (SiC) based materials and composites are well known for their high mechanical stability at high temperatures, thermal conductivity, excellent oxidation and corrosion resistance combined with a low density. Biomorphous carbide ceramics are interesting candidates for advanced engineering applications due to their anisotropic cellular structures [16]. The anisotropic mechanical properties of these materials were thoroughly investigated [33–36]. Biomorphous ceramics exhibit a higher strength at similar porosities with respect to conventional materials due to their uniaxial directed cellular microstructure. However, carbide ceramics usually show brittle failure. In our work, we used the uniaxial pore system of the rattan palm (*Calamus rotang*) to improve the mechanical properties of the corresponding biomorphous SiC ceramic, figure 4 [37]. First, samples of the rattan palm were pyrolysed to obtain a carbon template with the original plant microstructure. The carbon templates were then transformed into a SiSiC composite by infiltration with liquid silicon at 1450°C.

The large vessels (200  $\mu\text{m}$ ) of the original rattan template remained open after the ceramic conversion, whereas smaller cells were closed. The open vessels were subsequently filled with an aluminium silicon alloy (Al-Si) in a squeeze casting process. The developed technology can be applied to all cellular biomorphous ceramics. The resulting material is a SiSiC/Al-Si composite. Exemplarily, we extended our method to a complex form made from cardboard, figure 4d) [38].

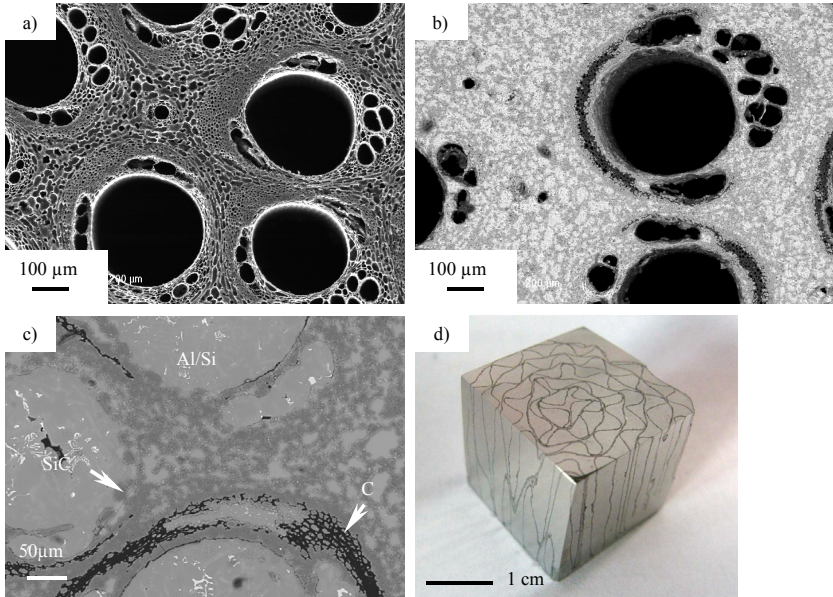


Figure 4: a) Microstructure of the rattan carbon template, b) corresponding biomorphous SiSiC ceramic, c) biomorphous SiSiC/Al-Si composite (Zollfrank et al. [37]; d) SiSiC/Al-Si composite obtained from rolled cardboard as biotemplate [36].

The biomorphous structure revealed a high amount of plastic deformation before the final failure. Analysis of the crack propagation showed, that the crack followed the original cell anatomy. Detachment and plastic deformation of the Al-Si alloy in the SiSiC matrix lead to an enhanced degree of crack deflection. Due to the pullout effect of the Al-Si alloy these biomorphous ceramics showed graceful failure behaviour. The rattan derived biomorphous SiSiC/Al-Si composite showed an average strength of 200 MPa, which is in the same order of magnitude as conventionally prepared SiC/Al-Si composites [38].

#### 4 Bioinspired materials using microalgae templates

Another approach for the formation of biotemplated inorganic materials is the use of polysaccharide structures provided by microorganisms. Several algae

species and cyanobacteria display a biopolymer layer on their cell surface which primarily consists of polysaccharides, the so-called exopolysaccharides (EPS, [39]). Glucuronic and galacturonic acids are responsible for the anionic character of the EPS next to sulphate and phosphate groups. Anionic EPS play an important role in biomineralisation processes [6]. In the present work, the red microalgae *Porphyridium purpureum* was used as a structural biotemplate. *P. purpureum* is found in nature in humid environments (bricks and soil) but also in open waters [40]. The cells have a spherical appearance with a diameter of approximately 5  $\mu\text{m}$  and they often appear in colonies or in small groups, figure 5.

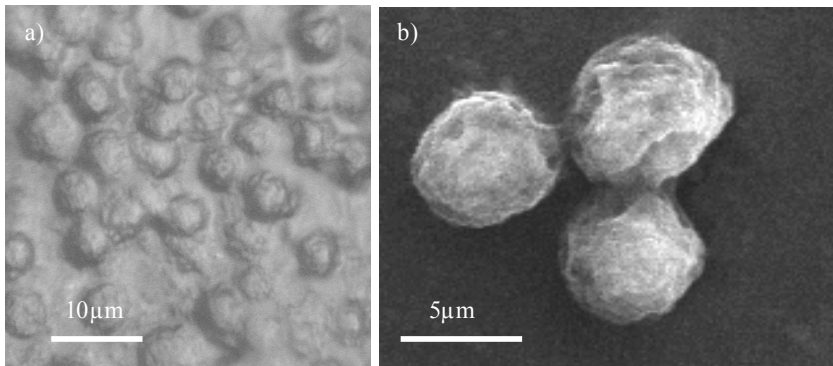


Figure 5: *Porphyridium purpureum*: a) colony and b) a group of three.

It is an interesting feature of *P. purpureum*, that the microalgae cells exhibit a certain mobility stimulated by light illumination. The movement of the microalgae is an answer on the light stimulus and is known as phototaxis [41]. The sliding movement of the cells is provoked by secretion of EPS. In inverse driving force acts on the cells, which result in a movement of the cells towards the direction of the illumination source. The observed velocities range from 0.004-10  $\mu\text{m/s}$  dependent on the species. Our novel concept involves the illumination of the microalgae through a mask, which forces the algae to reorganise according to the predefined pattern, figure 6.

After a few days, the generated pattern of microalgae exhibited a line width of approximately 500  $\mu\text{m}$ , which means that roughly 100 individual cells were building one line. The microalgae pattern and the secreted EPS are subsequently transformed into inorganic materials by sol-gel chemistry [42]. It was shown, that the microalgae could be completely covered with the inorganic sol. The thickness of the gel layer varied linearly with the sol concentration, figure 6c). Removal of the organic microalgae template by calcination between 450 and 600 $^{\circ}\text{C}$  yielded a mixture of  $\text{Pb}(\text{Zr},\text{Ti})\text{O}_3\text{-ZrO}_2\text{-PbO}$ . The diameter of the PZT coated microalgae was decreased by approximately 15% after calcination, which is due to the decomposition of the organic materials and biopolymers up to this

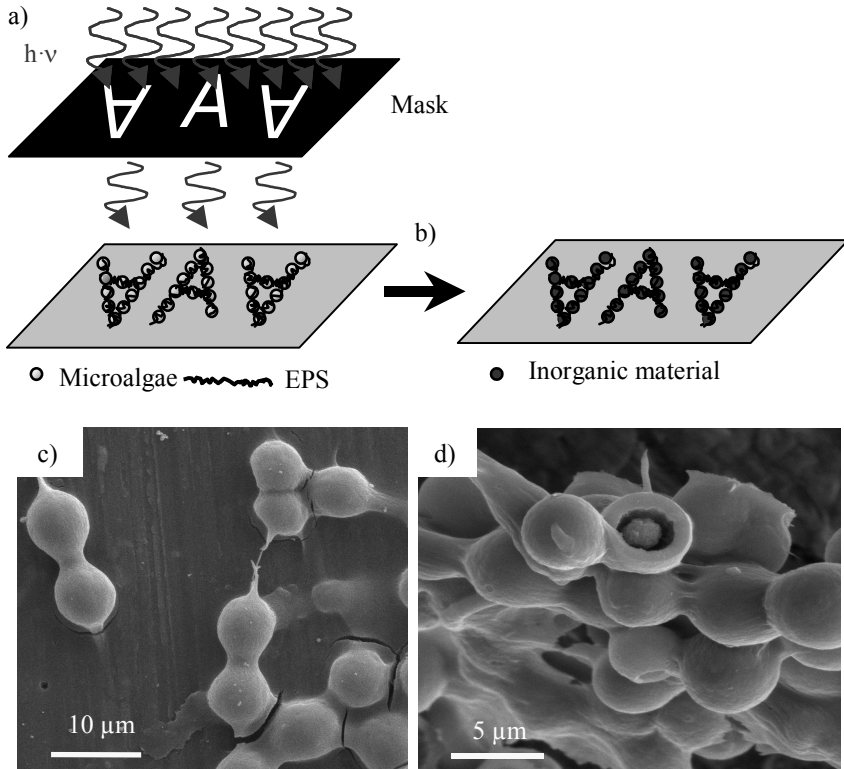


Figure 6: a) Concept for the light-induced patterning of microalgae and b) transformation of the patterned microalgae into an inorganic material by sol-gel processing, c) lead zirconate/ titanate (PZT) sol-gel-coated microalgae and d) calcined biotemplated and porous PZT spheres.

temperature, figure 6d). PZT thin films were prepared on gold-coated silicon substrates using sol-gel processing for dense films and microalgae templates. The dielectric properties of the resulting PZT films were investigated applying an alternating current of 100 mV at varying frequencies. The observed dielectric characteristics of the porous PZT using microalgae template films are appealing for piezoelectric applications in fluid media.

## 5 Summary and conclusions

The potential of selected polysaccharide biotemplates for the bioinspired formation of inorganic functional materials was demonstrated. It could be shown, that the polysaccharides are suitable templates in their natural (microalgae, plant tissue) or processed forms (cellulose whiskers) to generate a variety of inorganic materials. Because of the structural diversity, self-assembly



capabilities and derived processed materials, polysaccharides can be used on all length scales from the nano to the macro scale to fabricate structured inorganic materials. The polysaccharide cellulose is an exceptional template, not only because it is the most abundant biopolymer on earth ensuring continued availability but also because of large scale engineering of cellulosic products (e.g. paper, cardboard). Additionally, cellulose is more resistant towards acid hydrolysis and temperature treatment compared to many other synthetic and biopolymers (e.g. proteins). This results in an enlarged processing window to access inorganic materials, where less gentle chemical methods can be applied. The hydroxyl functional of the cellulose can be selectively functionalised to direct materials formation. The hydroxyl groups show self-assembling capabilities through the formation of a (reversible) hydrogen-bonding network ultimately resulting in the formation of hierarchically structured biotemplates. The use of living microalgae templates was introduced as a novel concept for the light-directed patterning of inorganic materials structures. The high potential of polysaccharides biotemplates in bioinspired materials synthesis still holds many unexplored routes for a multifunctional design of inorganic materials. It is most interesting to transfer biological features such as adaptation, self-organisation, self-healing or energy autonomy into engineering materials (self-x materials). The exploration of bioinspired materials design by biotemplating, however, requires the multidisciplinary cooperation of biologists, physicists, chemists and engineers.

## References

- [1] Sarikaya, M. & Aksay I.A., *Biomimetics: Design and Processing of Materials*. AIP Press: Woodbury, New York, 1995.
- [2] Greil, P., *Advanced engineering ceramics. Adv. Eng. Mater.*, **4**, pp. 247-254, 2002.
- [3] Mann, S., *Chemistry of Form. Angew. Chem.* **112**, 3532-3548, 2000.
- [4] Aizenberg, J., Livages, L. & Mann, S., (eds.) *New developments in bio-related materials. J. Mater. Chem.* **14**, pp. 2059-2354, 2004.
- [5] a) K. Naka (ed). *Biom mineralization I: Crystallization and Self-Organization Process. Topics Curr Chem.* **270**, 2007; b) K. Naka (ed). *Biom mineralization II: Mineralization Using Synthetic Polymers and Templates. Topics Curr Chem.* **271**, 2007.
- [6] Bäuerlein E., (ed.). *Biom mineralization*, Wiley-VCH: Weinheim, 2000.
- [7] Klaus-Joerger, T., Joerger, R., Olsson, E. & Granqvist, C.G., *Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trends Biotechnol.*, **19/1**, pp. 15-20, 2001.
- [8] Förster S. & Plantenberg T., *Von selbstorganisierenden Polymeren zu Nanohybrid- und Biomaterialien. Angew. Chem.*, **114**, pp. 712-739, 2002.
- [9] Cölfen, H. & Mann, S., *Geordnete mesoskopische Strukturen durch Selbstorganisation und Transformation von Hybrid-Nanostrukturen, Angew. Chem.*, **115**, 2 pp. 452-2468, 2003.



- [10] van Bommel K.J.C., Friggeri A. & Shinkai S., Organische Template zur Formgebung anorganischer Materialien. *Angew. Chem.*, **115**, pp. 1010-1030, 2003.
- [11] Lowe, C.R., Nanobiotechnology: The fabrication and application of chemical and biological nanostructures. *Curr. Opin. Struct. Biol.*, **10**, pp. 428-434, 2000.
- [12] Fan, T.X., Chow, S.K., & Zhang, D., Biomorphic mineralization: From biology to materials. *Prog. Mater. Sci.*, **54**, pp. 542-659, 2009.
- [13] Fratzl, P. & Weinkamer, P., Nature's hierarchical materials. *Prog. Mater. Sci.*, **52**, pp. 1263-1334, 2007.
- [14] Zollfrank, C., Bioinspired ceramics from polysaccharide templates, *Habilitation thesis*, Faculty of Engineering: University of Erlangen-Nuremberg, 2009.
- [15] Fratzl, P., Biomimetic materials research: what can we really learn from nature's structural materials? *J. R. Soc. Interface*, **4**, pp. 63-642, 2007.
- [16] Greil, P., Biomorphous ceramics from lignocellulosics. *J. Eur. Ceram. Soc.*, **21**, pp. 105-118, 2001.
- [17] Dujardin. E. & Mann, S., Bio-inspired materials chemistry. *Adv. Mater.* **14**, pp. 775-788, 2002.
- [18] Sotiropoulou, S., Sierra-Sastre, Y., Mark, S.S. & Batt, C.A., Biotemplated Nanostructured Materials. *Chem. Mater.*, **20**, pp. 821-834, 2008.
- [19] Hall, S.R., Biotemplated syntheses of anisotropic nanoparticles. *Proc. R. Soc. A*, **465**, pp. 335-366, 2009.
- [20] Hermans J., Flow of gels of cellulose microcrystals. I. Random, and liquid crystalline gels. *J. Polymer Sci. Part C*, **2**, pp. 129-144, 1963.
- [21] Revol, J.F., Godbout, L., Dong, X.M., Gray, D.G., Chanzy, H. & Maret, G., Chiral nematic suspensions of cellulose crystallites; phase separation and magnetic field orientation. *Liq. Cryst.*, **16**, pp. 127-134, 1994.
- [22] Revol, J.F., Godbout, L. & Gray, D.G., Solid self-assembled films of cellulose with chiral nematic order and optically variable properties. *J. Pulp Pap. Sci.*, **24**, pp. 146-149, 1998.
- [23] Heux, L., Dinand, E. & Vignon, M.R., Structural aspects in ultrathin cellulose microfibrils followed by <sup>13</sup>C CP-MAS NMR. *Carbohydr. Polym.*, **40**, pp. 115-124, 1999.
- [24] Helbert, W., Nishiyama Y., Okano T. & Sugiyama J., Molecular Imaging of, *Halocynthia papillosa* Cellulose. *J. Struct. Biol.*, **124**, pp. 42-50, 1998
- [25] Sugiyama, J., Chanzy, H. & Revol, J.F., On the polarity of cellulose in the cell wall of *Valonia*. *Planta*, **193**, pp. 260-265, 1997.
- [26] Dong, X.M., Revol, J.F. & Gray, D.G., Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose. *Cellulose*, **5**, pp. 19-32, 1998.
- [27] Bondeson, D., Mathew, A. & Oksman, K., Optimization of the isolation of nanocrystals from microcrystalline cellulose by acid hydrolysis. *Cellulose*, **13**, 171-180, 2006.
- [28] Uddin, M.J., Cesano, F., Bonino, F., Bordiga, S., Spoto, G., Scarano, D. & Zecchina, A., Cotton textile fibers coated by Au/TiO<sub>2</sub> films: Synthesis,



- characterization and self cleaning properties. *J. Photochem. Photobiol. A: Chem.*, **189**, pp. 286-294, 2007.
- [29] Nelson, K. & Deng, Y., The shape dependence of core-shell and hollow titania nanoparticles on coating thickness during layer-by-layer and sol-gel synthesis. *Nanotechnology*, **17**, pp. 3219-3225, 2006.
- [30] Dujardin, E., Blaseby, M. & Mann, S., Synthesis of mesoporous silica by sol-gel mineralization of cellulose nanorod nematic suspensions. *J. Mater. Chem.*, **13**, pp. 696-699, 2003.
- [31] Zollfrank, C., Scheel, H. & Greil P., Regioselectively ordered silica nanotubes by molecular templating. *Adv. Mater.*, **19**, pp. 984-987, 2007.
- [32] Scheel, H., Zollfrank, C. & Greil, P., Luminescent silica nanotubes from cellulose whisker templates. *J. Mater. Res.*, **24**, 5, pp. 1709-1715, 2009.
- [33] Greil, P., Lifka, T. & Kaindl, A., Biomorphous cellular silicon carbide ceramics from wood: II. Mechanical properties. *J. Eur. Ceram. Soc.*, **18**, pp. 1975-1983, 1998.
- [34] Greil, P., Vogli, E., Fey, T., Bezold, A., Popovska, N., Gerhard, H. & Sieber, H., Effect of microstructure on the fracture behavior of biomorphous silicon carbide ceramics. *J. Eur. Ceram. Soc.*, **22**, pp. 2697-2707, 2002.
- [35] Varela-Feria, F.M., Martinez-Fernandez, J., de Arellano-Lopez, A.R. & Singh, M., Low density biomorphous silicon carbide: microstructure and mechanical properties. *J. Eur. Ceram. Soc.*, **22**, pp. 2719-2725, 2002.
- [36] Travitzky, N., Windsheimer, H., Fey, T. & Greil, P., Preceramic paper-derived ceramics. *J. Am. Ceram. Soc.*, **91**, pp. 3477-349, 2008.
- [37] Zollfrank, C., Travitzky, N., Sieber, H., Selchert, T. & Greil, P., Biomorphous SiSiC/Al-Si ceramic composites manufactured by squeeze casting: Microstructure and mechanical properties. *Adv. Eng. Mater.*, **7**, pp. 743-746, 2005.
- [38] Ahlatci, H., Candan, E. & Cimenoglu, H., Abrasive wear behavior and mechanical properties of Al-Si/SiC composites. *Wear*, **257**, pp. 625-632, 2004.
- [39] Arad, S., Biochemicals from unicellular red microalgae. *Int. Ind. Biotechnol.*, **6**, pp. 281-284, 1987.
- [40] van den Hoek, C., Jahns, H.M. & Mann, D.G., *Algen*, Thieme: Stuttgart, 1993.
- [41] Häder, D.P., Photosensory behavior in procaryotes. *Microbiol Rev.*, **51**, pp. 1-21, 1987.
- [42] Steinau, M., Formation of functional ceramics on biotemplates, *Diploma Thesis*. Faculty of Engineering: University of Erlangen-Nuremberg, 2005.

