

Hydroxyapatite Growth on Glass/CdSe/SiO_x Nanostructures

L. Pramatarova¹, E. Pecheva¹, D. Nesheva¹, Z. Aneva¹, A. L. Toth²,
E. Horvath², F. Riesz²

¹ Institute of Solid State Physics, Bulgarian Academy of Sciences,
72 Tzarigradsko Chaussee blvd., 1784 Sofia, Bulgaria

² Research Institute for Technical Physics and Materials Sciences,
Hungarian Academy of Sciences, 29-33 Konkoly-Thege str., 1121 Budapest, Hungary

Key words: hydroxyapatite, growth from solution, CdSe/SiO_x nanostructure

Abstract. The aim of this study was to find if nanocrystal layers obtained by well-established nanotechnology are able to induce deposition of hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂]. It is known that nanosized objects and porous structures influence biological events and they may be used to create biologically integrated multifunctional devices including biomaterials and sensors. In this work, sequential physical vapour deposition of CdSe and SiO, or SiO_x film was used to modify glass substrates. To study the ability of the nanostructured surfaces to induce hydroxyapatite deposition, samples were immersed in a simulated body fluid and simultaneously irradiated with a scanning laser beam for a few minutes. This resulted in a porous sponge-like non homogeneous hydroxyapatite layer, consisting of networks of aggregates of nano dimensions on the modified surfaces. Analysis showed higher Ca and P contents in the stripes of the laser-substrate interaction, which indicated the influence of the laser energy. The method of laser-liquid-solid interaction used has led to a synergistic effect due to the simultaneous use of the nanostructured substrate, aqueous solution and laser energy.

Introduction

In nature, the inorganic material hydroxyapatite (HA) and collagen form bone - a complex but perfect nanocomposite. For many years, scientists have tried to mimic the process of biological mineralization to make such structures. Although much is known there is still much more to know. Recently nanotechnology has been used to fabricate nanotopography and nanopatterned surfaces. Nanotopography is important in a wide variety of biotechnological devices or biomedical applications. To date little work has been done to apply nanostructures as templates for HA growth. In this paper, a positive answer is given to the question whether a well-established technology, physical vapour deposition, could be efficiently used to obtain nanocrystal layers able to induce HA formation. The laser-liquid-solid interaction (LLSI) used promoted and enhanced the HA nucleation and growth compared to prolonged biomimetic soaking in a simulated body fluid (SBF).

Experimental

Sequential physical vapour deposition was used to deposit 31 layers of CdSe and 32 layers of SiO, each 5 nm thick. The structures were deposited on Corning glass substrates at a vacuum of 10⁻³ Pa (samples S47). The total thickness of the CdSe/SiO_x (x ≈ 1.5) multilayer structure obtained was 320 nm. A second group of samples (S101) was prepared at the same deposition conditions but with 15 layers of CdSe and 16 layers of SiO, each 2.5 nm thick. The total thickness of the structure was 80 nm. Subsequent annealing in air at 100⁰C for 60 min (S47) or 400⁰C for 90 min (S101) was used to improve the crystallinity of the CdSe layers [2]. A layer of SiO_x (x ≈ 1.5) with a thickness of 1 μm was grown on a glass substrate by evaporation of SiO at low vacuum and subsequent two-stage annealing in vacuum at 120⁰C for 60 min, and in air at 120⁰C for 240 min (samples S79) [2]. The

modified surfaces were immersed in SBF prepared from reagent-grade chemicals NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, Na₂SO₄·10H₂O, and CaCl₂·6H₂O dissolved in distilled water and buffered at pH 7.4. The Ca and P concentrations were 1.5 times higher than those used by Kokubo [3]. Furthermore, a novel method for the HA growth was applied, namely laser-liquid-solid interaction (LLSI) [4]. It includes simultaneous immersion of the samples in the fluid and irradiation by a pulsed laser (CuBr, $\lambda = 578,2$ nm, output power 330 mW, power density 50 MW/cm²) focussed on the substrate surface. Using scanning, a surface design of seven squares of 200 μm was formed at the end of each sample. The centre of the substrate was not irradiated. The interaction time was approx. 2 min. After irradiation the samples were soaked in the SBF at 37⁰C for 18 h, and they were then washed in distilled water and air dried. The morphology and the composition of the HA layers were investigated by using SEM (JSM-25 SIII) and EDX (JEOL JSM-840). FTIR spectra were recorded in a reflection mode and in a spectral range 4000-400 cm⁻¹, with a resolution of 4 cm⁻¹ (Nicolet Magna IR 750). Raman spectra were obtained by a Raman Micro-Spectrometer HR800 (Jobin Yvon Horiba).

Results and discussion

The SEM study of all samples at low magnification showed a sponge-like layer, non-homogeneously covering the surfaces. At higher magnifications (> 50 000 x) a porous network of aggregates having nano dimensions was observed and on this base overgrowths were formed (Fig. 1a,b). The samples from the group S79 induced the formation of a layer with higher roughness. Due to the higher number of overgrowths on these samples, the underlying network was hardly visible (Fig. 1c). The thick SiO_x layer (1 μm) was deposited in a few steps and was much rougher than the CdSe/SiO_x structures. It is considered that the subsequently grown HA repeated the topography of the SiO_x layer. Comparison was made with our previous work on plane, ion-implanted or protein-coated glass surfaces, where sphere-like aggregates were found forming the porous network [5,6]. The different morphology observed was assigned to the modification of the surfaces by nanocrystal CdSe or SiO_x layers. Raman spectra measured on the three samples (Fig. 2a) clearly showed the formation of HA layers. The spectra were dominated by the strong peak of ν_1 PO₄ stretching mode at 961 cm⁻¹. The two weaker peaks at 435 and 600 cm⁻¹ were considered as envelopes of ν_2 and of ν_4 PO₄ bending modes. The ν_3 PO₄ mode also contributed to the spectra in the region of 1040-1080 cm⁻¹ with weak peaks. A low intensive peak at 207 cm⁻¹ for sample S47 could be due to the 1LO CdSe mode, usually at 210 cm⁻¹ [7]. This mode was expressed only in this group because of the higher total CdSe thickness in this structure (160 nm) and/or the greater thickness of the less disordered individual CdSe layers. The peaks at 560 and 600 cm⁻¹ (ν_4 P-O bending), and that at 1022 cm⁻¹ (ν_3 P-O asymmetric stretching) observed in FTIR (Fig. 2b) were characteristic of HA. P=O stretching vibration appearing at 1240 cm⁻¹ in all samples.

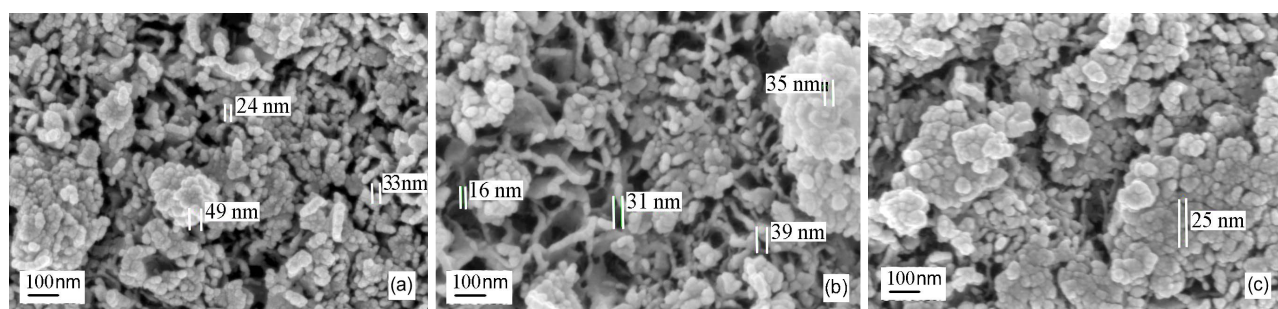


Fig. 1. SEM images of the layers grown by applying the LLSI method in SBF on samples: (a) S47; (b) S101 and (c) S79.

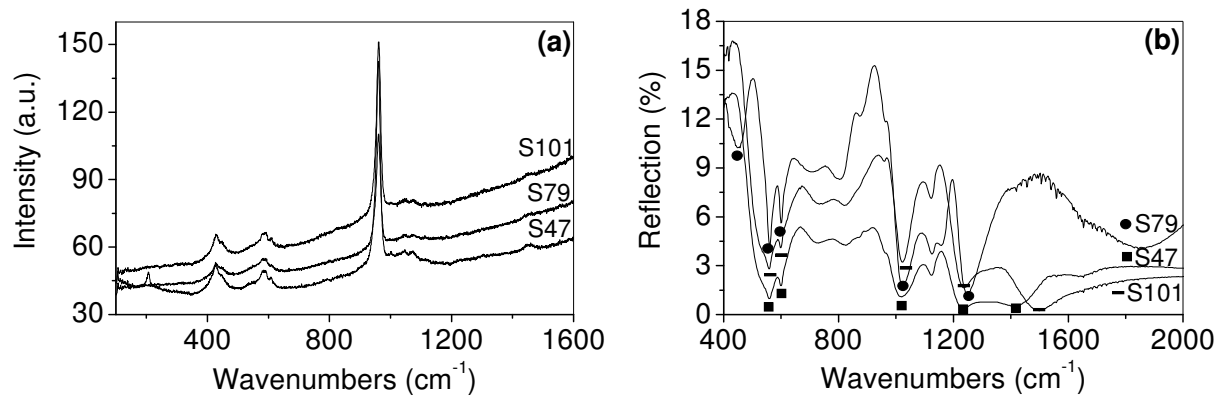


Fig. 2. (a) Raman and (b) FTIR spectra of the grown HA layers with applied LLSI in the SBF.

The peaks at 1419.3 cm^{-1} (S47) and 1498.4 cm^{-1} (S101) were assigned to C-O stretching mode in CO_3^{2-} , indicating CO_3 incorporation in the HA structure. IR absorption of the underlying SiO_x layers was also observed as Si-O-Si bending and stretching modes in the range of $730\text{--}960\text{ cm}^{-1}$ and $1120\text{--}1160\text{ cm}^{-1}$, respectively. A well-pronounced peak due to Si-O-Si rocking vibrations was observed at 450 cm^{-1} in the spectrum of sample S79. The peaks at 1860 and 2700 cm^{-1} in S79 could be assigned to C=C and C-H stretching modes. C-H stretching in the S47 and S101 samples was observed at 2840 cm^{-1} . Vibrations due to the O-H stretching of OH^- were detected at $3500\text{--}3550\text{ cm}^{-1}$ for all samples. The analysis of the FTIR data showed that the thickest HA layer was grown on the samples with the thicker CdSe/ SiO_x multilayered structure (S47), followed by the layer on samples S101. The samples with the evaporated SiO_x (S79) induced the thinnest HA layer. EDX results showed the presence of Ca, P, O, Si, Cd and Se in the layer, minor amounts of Mg and Na (Fig. 3), and a higher P than Ca concentration. The stripes of the laser irradiation were clearly visible on S47 and allowed measurement of Ca and P concentration and calculation of the Ca:P ratio in three areas.

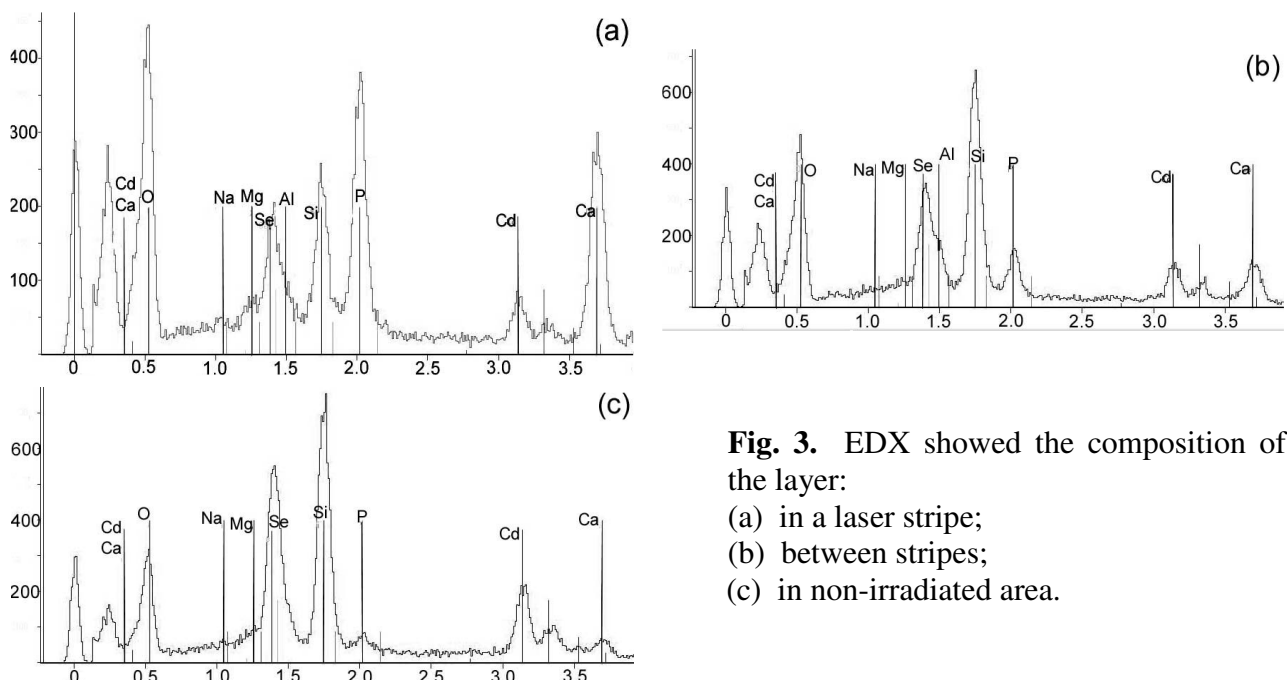


Fig. 3. EDX showed the composition of the layer:

- (a) in a laser stripe;
- (b) between stripes;
- (c) in non-irradiated area.

The highest Ca:P ratio of 0.76 was calculated in the stripes of the laser irradiation (Fig. 3a). It decreased to 0.61 between the stripes (Fig. 3b) and to 0.5 in non-irradiated areas (Fig. 3c). It is assumed that optical stimulation of the photosensitive CdSe is responsible for the higher concentrations of Ca and P in the laser stripes. CdSe in the prepared samples had an optical bandgap

of about 2 eV. On illumination with intense laser radiation (50 MW/cm^2 ; 578.2 nm that is equal to 2.2 eV), electron-hole pairs were photogenerated in the CdSe layers of the CdSe/SiO_x structure. It is known that when photoexcited carriers are captured by structural defects they can charge them (if neutral) or cause secondary ionization [8]. This charge could stimulate the predominant attachment of Ca and P ions from the fluid to the irradiated areas, which further facilitates HA growth.

Natural HA contains CO₃, small amounts of Na, Mg, K, Cl and other elements and has a porous structure that facilitates transport of body liquids [9]. Biological apatites are non-stoichiometric, with low degree of crystallinity and a crystal size less than 200 nm. All these properties ensure a higher extent of dissolution than crystalline commercial ceramics [9]. By analyzing the SEM images of the experiment layers, the FTIR spectra and the EDX composition, it is concluded that the layer was mimicking the biological apatite. LLSI has led to a synergistic effect due to the simultaneous use of the nanostructured substrate, aqueous solution and laser energy.

Conclusions

Well-established technique based on physical vapor deposition was applied in this study to grow SiO_x layers and multilayers containing nanocrystalline CdSe on glass substrates. The prepared nanostructures were found able to induce the growth of a porous sponge-like HA layer. This layer consisted of network of aggregates with nanodimensions and was grown by applying a LLSI in SBF. The different morphology of the layers, i.e. dense sponge-like layer instead of sphere-like aggregates observed in our previous experiments was assigned to the modification of the surfaces by CdSe or SiO_x layers. FTIR and EDX studies showed that the porous HA layers were CO₃, Mg and Na-containing, thus mimicking the biological apatite. Optical stimulation of the photosensitive CdSe was probably the reason for the higher concentrations of Ca and P in the laser stripes. The LLSI has led to a synergistic effect due to the simultaneous use of the nanostructured substrate, aqueous solution and laser energy.

Acknowledgements

This work was supported by the Bulgarian National Scientific Research Fund, Grant L1213, and by a Deutsche Forschungsgemeinschaft, Germany. We thank Pulslight Ltd, Sofia for the laser equipment.

References

- [1] A. Curtis, C. Wilkinson: *Mater. Today*, May-June (2001), p. 22
- [2] D. Nesheva: *J. Optoelectr. Adv. Mater.* Vol. 3 (2001), p. 885
- [3] T. Kokubo et al.: *J. Biomed. Mater. Res.* Vol. 24 (1990), p. 721
- [4] L. Pramatarova, E. Pecheva, D. Nesheva, Z. Levi, Z. Aneva, R. Pramatarova, U. Bismayer, T. Petrov, *phys. stat. sol. (c)*. Vol. 0 (3) (2003), p. 1070
- [5] E. Pecheva, L. Pramatarova, M. Maitz, M. Pham, A. Kondyurin: *Appl. Surf. Sci.* (2004) in press
- [6] E. Pecheva, L. Pramatarova, M. F. Maitz, M. Pham, A. Kondyurin, *Ann. Transpl.* (2004) in press
- [7] D. Nesheva, C. Raptis, Z. Levi: *Phys. Rev. B*. Vol. 58 (1998), p. 7913
- [8] R. Bube, *Photoelectronic Properties of Semiconductors* (Cambridge University Press, 1992)
- [9] R. LeGeros, J. LeGeros: in *Phosphate Minerals*, J. Nriagu, P. Moore (eds.). (Springer Verlag, New York 1984)