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Carolina H Navarro, Karla J Moreno, Ana Arizmendi-Morquecho, Alejandra Chávez-Valdez and Santos García-Miranda

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Preparation and tribological properties of chitosan/hydroxyapatite composite coatings applied on ultra high molecular weight polyethylene substrate

Carolina H Navarro¹, Karla J Moreno¹, Ana Arizmendi-Morquecho², Alejandra Chávez-Valdez³ and Santos García-Miranda¹

Abstract
We prepared a chitosan/hydroxyapatite composite as coating on ultra–high-molecular-weight polyethylene substrates. The hydroxyapatite was prepared by a precipitation method obtaining particles with nanometer sizes with needle and spherical morphologies. The coating was prepared by a mixture of hydroxyapatite and lower molecular weight chitosan. Fourier transformed infrared spectroscopy technique was used to study the hydroxyapatite synthesis and composite coating. The chitosan/hydroxyapatite coating has been compared with a pure chitosan coating on ultra–high-molecular-weight polyethylene substrate in order to study the influence of hydroxyapatite incorporation. The characterization of coating thickness was performed by light microscopy, whereas the surface morphology was characterized by scanning electron microscopy. A ball-on-disc tribometer, employing a 6-mm diameter tungsten carbide ball, was run as a test method to

¹Instituto Tecnológico de Celaya, Guanajuato, México
²Cimav-Unidad Monterrey, Nuevo León, México
³Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Erlangen, Germany

Corresponding author:
Karla J Moreno, Instituto Tecnológico de Celaya, Apartado Postal 57, 38010 Celaya, Guanajuato, México
Email: moreno_bello_karla@yahoo.com.mx
investigate tribological behaviors of the chitosan/hydroxyapatite and chitosan coatings in dry conditions. It was found that the friction coefficients of the chitosan/hydroxyapatite coating have lower values than chitosan coating. The chitosan/hydroxyapatite coating exhibited lower wear rate values than the chitosan coating. The incorporation of hydroxyapatite nanoparticles into chitosan increased the coating thickness from 20 μm to 37 μm. We have found a composite hardness of 9.1 HV and 6.4 HV for the chitosan and chitosan/hydroxyapatite coating, respectively.

**Keywords**
Composite, coating, chitosan-hydroxyapatite, wear, hardness

**Introduction**
A wide range of biomaterials for different biomedical applications can be prepared on the basis of two components, chitosan (CS) and hydroxyapatite (HA), due to their excellent features of bioactivity, biocompatibility. CS is a natural biodegradable polymer and is a derivate of chitin. It was suggested as an alternative polymer for orthopedic applications in order to provide temporary mechanical support for the regeneration of bone cell in growth due to its unique properties, including biocompatibility, nontoxicity, biodegradability, inherent wound healing characteristics and an antimicrobial effect. Also, it has been reported that CS promotes the growth and differentiation of osteoblasts in culture to enhance osteogenesis. Therefore, this material is used for biomaterials applications such as artificial skin, wound dressings and sutures. CS has been used in various forms such as zero dimension microsphere, two-dimension membrane, three-dimension pin or rod and also as polymer matrix of mica clay nanoparticles in the preparation of nanobiocomposites showing a good dispersion of the fillers maintaining its biodegradability.

Natural bone is a complex inorganic-organic nanocomposite material, in which HA (Ca_{10}(PO_{4})_{6}(OH)_{2}) and collagen fibrils are well-organized in a hierarchical architecture over several length scales. HA has been used in the biomedical fields due to its ability of accelerating the bone-like formation on the surface of implants, and also due to its biocompatible and osteoconductive nature. Composites comprising calcium phosphates and natural biopolymers are widely used as biomaterials for bone tissue repair and engineering. CS can be utilized in combination with other bioactive inorganic ceramics, especially HA, to further enhance tissue regenerative efficacy and osteoconductivity. Incorporation of HA with CS, the mineral component of bone, could improve the bioactivity and the bone-bonding ability of the
chitosan/hydroxyapatite (CS/HA) composites. Therefore, it is desirable to develop a composite material which takes the favorable properties of both CS and HA materials. The designed composite is expected to show increased osteoconductivity and biodegradation together with sufficient mechanical strength, which will be of great importance for future applications. Several works have reported the preparation of chitosan-hydroxyapatite mixtures in the form of powder, membranes, pastes, cements, scaffolds or microspheres; however, they do not deal with the performance as a coating. The goal of this work is to obtain a composite of CS/HA in the form of a solution and evaluate it as coating on ultra high molecular weight polyethylene (UHMWPE) substrates. We analyzed the influence of adding hydroxyapatite into CS coating on the morphology, surface hardness and wear behavior of CS coating.

**Experimental section**

**Chemical and instrumentation**

CS with the deacetylation degree of 92% and low molecular weight (C\textsubscript{12}H\textsubscript{24}, \(M_t = 5.89 \times 10^4\) Sigma Aldrich), phosphoric acid, reagent grade crystalline (H\textsubscript{3}PO\textsubscript{4}, 99% Sigma Aldrich), acetic acid (CH\textsubscript{3}COOH, 99.8%) and distilled water were used as received. Calcium oxide (CaO) previously prepared\textsuperscript{30} was used to obtain HA. The pH value of suspension was monitored by a pH meter from Denver Instrument. The formation of the HA phase was evaluated by Fourier transformed infrared spectroscopy (FTIR) spectral analysis. FTIR transmittance spectra of the samples were obtained in the 4000–400 cm\textsuperscript{-1} region in a Nicolet 6700 with a coupled DRIFT cell. The phase obtained was also identified by X-ray diffraction (XRD) using a Philips X-PERT Model 95 PW3090 diffractometer with a nickel-filtered Cu-K\(\alpha\) radiation operated at 40 kV and 30 mA, in the 2\(\theta\) range of 15° to 80° at a rate of 0.02°/min. The morphology, size and composition of the synthesized HA powder were analyzed by scanning electron microscopy (SEM) with scanning transmission electron microscopy (STEM) mode in an FEI Nova nano SEM 200 equipped with an energy dispersive X-ray (EDAX) detector for microanalysis. The samples were prepared by drying the solution of HA on a copper grid fitted with a carbon support film. The solution was prepared by dispersing HA-synthesized powder in acetone with an ultrasonic processor.

**Synthesis of HA**

Hydroxyapatite was obtained using a modified version of a precipitation method\textsuperscript{31} by the reaction between calcium hydroxide (Ca(OH)\textsubscript{2}) suspension and H\textsubscript{3}PO\textsubscript{4} solution at room temperature. Under rigorous stirring, an aqueous
solution of reagent grade phosphoric acid (1.8 M) was added in drops at a controlled rate to a stoichiometric solution (3.0 M) of calcium oxide dispersed in distilled water previously stirred for 5 h. After complete addition, the solution remained in constant stirring for 24 h, and then it was subjected to ripening (aging) treatment for 24 h followed by 1 h refluxing at 90°C. It was then stirred for another 1 h without heating and left for 48 h. The precipitate was filtered and thoroughly washed with distilled water and filtered again. After drying at 120°C for 4 h, the precipitate was calcined at 800°C for 2 h.

Chitosan/hydroxyapatite composite synthesis

The CS solution was prepared with 20 ml of acetic acid, 10 ml of distilled water and 105 mg of CS. The solution was stirred for 3 h. After that, a homogeneous solution was obtained. Then, 60 mg of HA were added to this solution and stirred for 14 h to obtain the CS/HA solution. This solution showed high stability, that is, it did not precipitate spontaneously within a time span of 2 weeks after preparation.

Preparation of coatings

UHMWPE substrates with dimensions 25 mm × 25 mm were polished to a surface roughness $R_a \leq 0.5 \mu m$. The coating was prepared by immersion of UHMWPE substrates in the CS/HA solution. The dipping was performed by using a mechanical device. The immersion speed was 70 mm/min for 5 min. After dipping, the samples were slightly dried in air for 10 min. Subsequently, the coated substrates were dried at 120°C for 3 h. For comparative studies, the same methodology was applied to prepare CS coatings.

Coatings characterization

Thickness estimation was analyzed by light microscopy in an Olympus GX-51 microscope. The surface morphology was characterized by using a FEI Nova nano SEM 200. The surface roughness $R_a$ of the coatings and UHMWPE substrate were analyzed using a MITUTOYO Surftest 402, twenty measures were made in perpendicular direction for each coating in an area of 40 cm².

Wear and hardness tests

Wear tests were carried out on CSM Instruments Tribometer by pin-on-disk test in dry conditions. The kinetic friction coefficient ($\mu_k$) was obtained directly of the Tribox 4.1 software. Tungsten carbide (WC) ball with a diameter of 6 mm, with a roughness $R_a = 0.05 \mu m$ and the surface hardness of
2300 HV was previously cleaned with alcohol and slide on the UHMWPE substrate already coated with the CS/HA composite. The WC ball was fixed on the load arm and the UHMWPE test tube rotated by an electrical motor controlled by a frequency converter. In all wear tests, the contact loads were 2, 4, 6 and 10 N. The sliding speed was 0.10 m/s, with a total distance of 300 m. UHMWPE coated with CS was also analyzed in order to compare the tribological behavior between them. The volume loss values were determined by a standard test method by ASTM G99\textsuperscript{32} using the following equation in accordance with Polok-Rubinie\textit{c} et al.\textsuperscript{33} assuming that there is no significant pin wear:

\[
V = (\pi RD^3)/6r
\]  

where \(V\) is the wear volume (mm\(^3\)), \(R\) is the friction radius (mm), \(D\) is the wear trace width (mm) and \(r\) is the ball radius (mm).

The wear rate \(k\) was determined according to the equation given in ref. 34:

\[
k = V/Lx
\]  

\(V\) is the wear volume (mm\(^3\)), \(L\) is the load (N), \(x\) is the sliding distance (m) and \(k\) is the wear rate (mm\(^3\)/Nm). The microsurface hardness of CS and CS/HA coatings on UHMWPE were evaluated by using a Vickers indenter. In order to discard any substrate contribution in the hardness coatings, indentation tests were also performed for UHMWPE substrates. Tests were carried out on a microhardness apparatus (Matsuzawa MMT-X7 with Clemex CMT Software) under indentation load of 98 mN. Ten indentations were made at each load, yielding twenty diagonal indentation measurements from which the average hardness was calculated by using the microhardness software. The theoretical depth indentation \((D_{th})\) was determined according to the equation presented by Chicot et al. for a Vickers pyramidal indenter, where \(d\) is the indent diagonal:\textsuperscript{35}

\[
D_{th} = d/7
\]

**Results and discussion**

**Formation of HA**

The pH measurements in the precipitation method to synthesize HA are very important in order to obtain a complete formation of HA phase. In our work, initially the pH of the 3.0 M calcium hydroxide suspension was 14. The pH was kept almost constant with the addition of 65 ml of \(H_3PO_4\). A further addition of \(H_3PO_4\) solution decreased drastically the pH of the Ca(OH)\(_2\)
suspension to 8 and increased the temperature from 26°C to 52°C. Finally, the suspension reached a pH = 5 after the complete addition of H$_3$PO$_4$ maintained a high temperature. After 2 min, the pH increased to 6 and the temperature decreased to 47°C. These changes in the reaction have been related with the formation of HA particles, where OH$^{-1}$ ions are supplied by the calcium hydroxide (Ca(OH)$_2$) solution, keeping a high value of pH and Ca$^{2+}$ ions and PO$_{3-}^{4}$ ions are added to form apatite particles. The increase of pH to 6 corresponds to the reaction of the remaining Ca(OH)$_2$. Figure 1(a) shows the X-ray diffraction pattern corresponding to HA synthesized using the precipitation method. The XRD pattern for HA exhibited reflections which are in agreement with those of the Joint Committee on Powder Diffraction Standards (JCPDS) card number 09-0432 (Figure 1(b)), proving the HA formation. No additional phases seem to be present in the powder. This XRD profile also shows broadening in the main reflections which suggest that the crystals have nanometer dimensions.

Figure 1. X-ray diffractogram of the hydroxyapatite produced by using the precipitated method compared with a Joint Committee on Powder Diffraction Standards (JCPDS) card number 09-0432 (hydroxyapatite; HA).
Figure 2 gives the FTIR spectra corresponding to HA, this spectra shows the characteristic absorption peaks of the PO$_{3}^{3-}$ and OH$^{1-}$ in the HA structure. The bands between 1100 cm$^{-1}$ and 650 cm$^{-1}$ are related with PO$_{3}^{3-}$ vibrations ions, whereas the band at 3570 cm$^{-1}$ is attributed to the vibrations of OH$^{1-}$ group in HA.\textsuperscript{37} Additionally, this spectrum contains bands at 1460 cm$^{-1}$ and 1415 cm$^{-1}$, which are associated with the CO$_{3}^{2-}$ group\textsuperscript{38} due to the vibration modes of carbonate ions. It is well-known that the biological apatite is commonly calcium deficient and is always carbonate substitute for phosphate groups and for this reason is generally called ‘carbonate apatite’ (CA).\textsuperscript{39} Then, the stoichiometric HA, which has a ratio of Ca/P = 1.67, differs from CA in its stoichiometry, however carbonate substitutions frequently alter HA structure and its properties, when such substitution takes place, HA is commonly referred to as ‘carbonate hydroxyapatite’ (CHA) with Ca/P ratio of 1.50.\textsuperscript{40} The chemical composition of the HA sample exhibited in Figure 1 was also examined by EDXS founding an average Ca/P ratio of 1.52. Thus, this indicates that the HA prepared in this work is a carbonate HA. It is also known that the presence of carbonate group in the HA structure increases its

![Figure 2. Fourier transformed infrared spectroscopy (FTIR) spectra of the hydroxyapatite synthesized.](image-url)
reactivity leading to a faster dissolution in acid environment. Therefore, CHA structures could be more attractive for medical applications and beneficial for future applications of this composite. On the other hand, FTIR spectra exhibited the presence of other bands around 2000 cm$^{-1}$, which can be related with the CO$_2$ present in the atmosphere due to the sensibility of the device.

Figure 3 shows SEM images with STEM mode of HA-synthesized powders. Figure 3(a) shows an agglomerate group of particles with nanometer sizes and irregular morphologies. It is difficult to identify which is the predominant morphology of the particles; however, we can observe the presence of spherical and needle-shaped particles whereas Figure 3(b) shows an isolated HA nanoparticle. The average of the length and width for needle-shaped particles was 158 nm and 64 nm, respectively. The average diameter size for spherical particles was 74 nm. Several works have mentioned that the morphology of HA particles can vary with the chemical and physical parameters of the precipitation methods, where the pH of the solution, aging time and temperature, as well as the molar ratio between Ca(OH)$_2$ and H$_3$PO$_4$, can affect the shape and size of the resultant particles. Tanaka et al. found out that an increase in the Ca(OH)$_2$ concentration causes a decrease in the particle size leading to the formation of particles with needle and spherical shape within a range of the length and width for needle-shaped particles of 50–100 nm and 20–40 nm, respectively, which are in agreement with our results.

**CS/HA composite characterization**

Figure 4 shows the FTIR spectra of CS/HA coating. This spectra shows broadening bands compared with the HA spectra, the bands observed at 1127 cm$^{-1}$ to 1700 cm$^{-1}$ in the spectra are associated with vibrations groups of amide I (C=O), amino (−NH$_2$) and amide II (−NH). The band at 1015 cm$^{-1}$ corresponds to the PO$_{3-4}^-$ ions of the HA structure while the broadening of the band at 1048 cm$^{-1}$ shows the presence of polymer and its interaction with phosphate groups. The strong band at 3361 cm$^{-1}$ is attributed to OH$^{1−}$ stretching vibrations in the acetic acid and it could also be related with the OH$^{1−}$ groups present in the HA structure.

**CS/HA coating evaluation**

Figure 5 shows SEM images of the CS (a) and CS/HA (b) coatings on UHMWPE with very distinctive surface morphology. CS coating exhibits a smooth surface characteristic of this polymer whereas the CS/HA coating showed a flake-like morphology, which is due to the incorporation of inorganic material that changed the surface of the polymer coating. The CS and CS/HA coatings had a surface roughness ($R_a$) of 0.3 ± 0.01 µm and
Figure 3. Scanning electron micrographs of synthesized hydroxyapatite nanoparticles showing (a) a group of nanoparticles and (b) isolated particle with characteristic needle morphology.
It is well known that surface roughness of the counter-surface is one of the main factors that influence the wear rate in UHMWPE components, to overcome that phenomenon in this study, the counter-surface was simulated by WC ball with a smoother surface; however, the increase in the surface roughness of the CS/HA coatings could have an influence on their tribological performance. The thickness estimation by image analysis shows an average thickness of 20 μm and 37 μm for CS and CS/HA coatings, respectively. The coatings did not break during the polishing process which may suggest a high adhesion to the substrate. In order to evaluate this hypothesis, the adhesion between coating and substrate was determined by a tape test according to the method indicated in the ASTM D3359-09 which is applied to evaluate the adhesion percentage between the interface of composite coatings and different substrates. CS/HA coating presented an area almost intact when the tape was stripped off showing a 95% of adhesion, while the CS coating exhibited approximately 90% of adhesion. It is important to mention that the adhesion test results could be affected by the surface roughness of the coatings.

Figure 6(a) and (b) shows typical kinetic friction coefficients versus sliding distance plots for CS and CS/HA coatings on UHMWPE. The friction coefficients of both coatings showed high variation at the initial stage (between 0 to 0.5 ± 0.02 μm, respectively.)
Figure 5. Surface micrographs of the pure chitosan (CS) coating (a) and chitosan/hydroxyapatite (CS/HA) coating (b).
Figure 6. Friction coefficients variations with respect to the sliding distance for chitosan (CS) (a) and chitosan/hydroxyapatite (CS/HA) (b) coatings on UHMWPE substrates against Tungsten carbide (WC).
and 100 m of sliding distance) and a decrease with the increasing load which could be related with an enhancement in contact area due to the soft substrate, but it was always higher for CS coating. CS coating exhibited in general stable friction coefficients behavior during the sliding distance; however, the friction coefficient pattern for a load of 10 N showed a slight fluctuation. Also, we can observe that the friction coefficients of CS coating were gradually decreased from 0.273 to 0.058 whereas the friction coefficients for CS/HA decreased from 0.113 to 0.051. Although, the CS/HA coating showed lower friction coefficients exhibited high fluctuations during the whole distance even at lower loads, more fluctuation at 2 N and lower friction coefficients can be seen. The fluctuations in the friction coefficients of CS/HA coating could be related to its surface roughness and its chemical composition, however, showed friction coefficients lower than an implanted UHMWPE with nitrogen ions, which exhibited friction coefficients of 0.26, 0.18 and 0.16 in dry friction at the sliding distance up to 300 m. On the other hand, the volume loss values are enlisted in Table 1 along with the average friction coefficients for each load. For both samples, friction coefficients decrease when the load increases due to the enhancement in contact area. It can be seen the volume loss values increase with the increasing load.

Figure 7 shows the wear rate values for the CS and CS/HA coatings on UHMWPE in bar charts for each load tested. It is shown that both samples have different changes in the wear rate with respect to the load. The wear rates of CS coating show the highest values in agreement with its high coefficient friction values. As it can be seen, the wear rate of CS/HA coating decreases one order of magnitude with respect to CS coating. Generally, polymers filled with nanoparticles show improvement in the wear resistance compared with the pure polymer, even up to three orders of magnitude. This has been related with several factors such as the reinforcement against abrasion due to the nanoparticles incorporation, the supporting load of the nanoparticles and the contribution to restrain the scuffing and adhesion of the polymer matrix.

### Table 1. Friction coefficient and volume loss for CS and CS/HA coatings on UHMWPE substrates

<table>
<thead>
<tr>
<th>Load (N)</th>
<th>( (\mu_k) )</th>
<th>((\mu_k))</th>
<th>Volume Loss (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.27 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>0.23 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.22</td>
</tr>
<tr>
<td>6</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.49</td>
</tr>
<tr>
<td>10</td>
<td>0.05 ± 0.03</td>
<td>0.05 ± 0.002</td>
<td>0.68</td>
</tr>
</tbody>
</table>

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On these basis, we speculate that the HA nanoparticles could restrain the wear mechanisms of the CS matrix in sliding against the tungsten carbide counter-surface and consequently the CS/HA composite can show better wear resistance than the pure CS. The lowest wear rate found in this study was for CS/HA at 2 N with $k = 4.47 \times 10^{-5}$; we note that this value is very close to that at 10 N with $k = 4.72 \times 10^{-5}$. The wear rates of CS/HA coating are comparable to those reported by Ge et al.\textsuperscript{50} of ions implanted UHMWPE sliding against ZrO\textsubscript{2} in dry friction with lower loads. Ge et al.\textsuperscript{50} also reported an increment of microsurface hardness of implanted UHMWPE of 9.3 to 15.3 HV at increasing ions concentration, whereas in our work the measured microsurface hardness was of 6.5 HV, 9.1 HV and 6.4 HV for UHMWPE substrate and CS and CS/HA coatings on UHMWPE, respectively. CS coating showed the highest hardness value. Thus, the CS/HA-coated UHMWPE and uncoated UHMWPE show similar hardness properties. Therefore, this concentration of HA may provide an effect in the molecular chains of the polymer, decreasing the hardness. Danilchenko et al.\textsuperscript{28} presented a study of solid samples of CS-HA composites where the Vickers hardness decreases gradually with the decreasing HA concentration for the composites with CS/HA weight ratios of 15/85 to 50/50 and hardness increases for a ratio of 80/20. The weight ratio of CS/HA
presented in this work is 64/36, which could have similar behavior in accordance with this ratios reported. The origin of this effect could involve a study of composites with different CS-HA concentration ratios and also different techniques such as Raman spectroscopy and Nuclear Magnetic Resonance. At this moment, the access at this technique is out of our possibility but is taken into account for the forthcoming works. The depth indentation \( D_{th} \) achieved in this test was 7.5 μm for UHMWPE substrate, 6.7 μm for CS and 7.5 μm for CS/HA. It is important to note that the hardness between UHMWPE substrate and CS/HA coating is almost similar as well as its indentation depth which could be confusing when analyzing the CS/HA coating hardness contribution. This could be explained in terms of the relative indentation depth, \( \beta \), given by the ratio between the indentation depth and the coating thickness. Values of \( \beta > 1 \) indicate that the indenter severely deformed and penetrated the coating reaching the substrate. Thus, the system response is dominated by the substrate properties. On the other hand, when \( \beta < 0.1 \), the influence of the substrate on the formation is negligible and only the coating response is observed. In our case, we have values of \( \beta = 0.20 \) and 0.33 for CS/HA and CS coating, respectively. Therefore, this suggests that the coatings hardness is influenced by the UHMWPE substrate. Thus, the CS and CS/HA coating hardness measurement should be considered as a composite hardness. Therefore, one solution is to separate the contribution the substrate makes to the hardness measurement can be to use a hardness thin film model. We may address this point in our future publication.

**Conclusions**

HA was successfully combined with CS to form a satisfactory coating on UHMWPE. Powder with nanometer HA was synthesized by precipitation and an FTIR study confirmed the formation of a typical ‘carbonate hydroxyapatite’ phase. Adding HA powder into CS increased the coating thickness. The CS and CS/HA coatings showed high percentage adhesion on UHMWPE substrates according to a standard test method. The CS/HA coating improves tribological performance. Within this study, the CS/HA coating showed better tribological performance at higher loads compared to other studies that proposed a surface treatment method in order to enhance the wear resistance of UHMWPE. These results could be considered preliminary studies for the appropriate evaluation of the tribological behavior and mechanical properties according to future applications.

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References
Biographies

Carolina H Navarro Carolina Navarro has a BS in Chemical Engineering from the Technological Institute of Celaya (ITC). She got the title of Master of Science in Chemical Engineering at the ITC in December 2010. She is currently doing a Doctoral thesis on the synthesis and characterization of materials with biocompatibles properties.

Karla J Moreno Karla Moreno has a BS in Chemical Engineering from the Technological Institute of Ciudad Madero (ITCM). She received her Master Degree of Science in Ceramic Engineering and her Doctor Degree in Science in Metallurgy and Ceramic Engineering from the Centre for Research and Advanced Studies of IPN (CINVESTAV-IPN). Her research is focused on the synthesis of different composites materials and their characterization for specific applications. She has authored several papers and she is the leader of Materials and Biomechanics System Group of the Mechanical Engineering Department in the Technological Institute of Celaya.

Ana Arizmendi-Morquecho Ana Arizmendi obtained a BS in Materials Engineering at the Institute of Technology Saltillo (ITS). She obtained the title of Doctor in Science in Metallurgy and Ceramic Engineering from CINVESTAV-IPN. She worked for 7 years in the industry in the area of research and development of new products. During the last 4 years, she has been working in projects focused on the synthesis of nanostructured coatings and multiphase surfaces in various types of materials at the Center for Advanced Materials Research S.C. (CIMAV, Monterrey Unit).

Alejandra Chávez-Valdez Alejandra Chávez has a BS in Chemical Engineering from ITCM. She received her Master degree of Science in Ceramic Engineering and her Doctor Degree of Science in Metallurgy and Ceramic Engineering from CINVESTAV-IPN. She currently attends a postdoctoral stay at the Institute of Biomaterials of the Department of Materials Science and Engineering in the University of Erlangen-Nuremberg in Germany. Her research is focused on electrophoresis method for materials with biomedical applications.

Santos García-Miranda Santos García has a BS in Electromechanical Engineering from the Autonomous University of Querétaro and a PhD in Mechanical Engineering from the University of Science and Technology of Lille in France. The areas of interest of Dr. García are the fatigue of materials and biomechanics, areas in which he has several publications in international journals.