Bioceramic nanoparticle/collagen nanofiber composites: A nanoindentation study

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ABSTRACT: The IREE supplement to current NSF award CMS-0555778 (PI: Stanishevsky) has been focused on the expansion of the current research program through the international collaboration with the partners at the Technical University of Lodz, Poland, and Technical University of Liberec, Czech Republic. This IREE supplement supported the visits, training, research, and cultural activities of the PI and four undergraduate female students (students stayed 2 months in Lodz and 1 month in Liberec). The results of these activities improved the knowledge about the microarchitecture evolution of the nanoparticle bioceramic/biopolymer fibrous composites, helped to obtain valuable information on the microarchitecture and mechanical properties of these composites and to optimize the precursor preparation techniques to fabricate the composites with higher level (up to 70 wt.%) of ceramic nanoparticle loading. The major broader impacts of this IREE program included ***(i)*** *the establishing of new partnerships between the PI on the current NSF award and the scientists from Ecole Catholique d'Arts et Métiers (ECAM) (Lyon, France), Technical University of Liberec (Czech Republic), Ecole Nationale Supérieure d'Arts et Métiers (ENSAM, Cluny Center, France), as well as the new perspectives for broader participation of UAB undergraduate and graduate students in the international research and student exchange programs, and* ***(ii)*** *the establishing of long-term collaborative activities between the Technical University of Lodz and UAB within the new NSF-funded Materials World Network project in the field of nanostructured carbons (PI: Stanishevsky, Co-PI; Vohra).*

INTRODUCTION

Major goal of the research conducted at the University of Alabama at Birmingham (UAB) under NSF award CMS-0555778 is to establish the correlation between the mechanical properties of bioceramic / biopolymer nanofiber composite materials and their microarchitecture and composition. The ongoing research program focuses, in part, on such aspects of these materials as ***(i)*** preparation of ion-substituted and surface-modified hydroxyapatite (HA) nanoparticles with controlled size, shape, and morphology; ***(ii)*** fabrication of HA nanoparticle-loaded biopolymer (collagen, gelatin) composite constructs with various microarchitectures; and ***(iii)*** characterization of the structure, morphology, and viscoelastic properties of the composites.

The rationale of this IREE trip was to expand the scope of the current program by introducing an international research and training component through the collaboration with established international research center specializing in the biomaterials and their applications, namely, EU-funded Centre of Excellence NANODIAM at the Institute of Materials Science and Engineering (IMSE), the Technical University of Lodz (TU-Lodz), Poland. The IREE Program provided a good opportunity for the PI, Dr. Stanishevsky, to renew the collaboration with the colleagues at TU-Lodz, and to use their facilities for more detailed studies of the developed materials. The primary contact person at TUL was Dr. Stanislaw Mitura, who is the Director responsible for Science in IMSE, and is the coordinator of the NANODIAM. To expand the experiences of the UAB IREE participants, the Polish partner proposed to extend the IREE program towards the research activities at the Laboratory of Materials Science, Technical University of Liberec (TU-Liberec, partner of NANODIAM), Czech Republic. Dr. Mitura holds a professor appointment

at TU-Liberec as well, and he also coordinated the IREE activities there together with Dr. Petr Louda, Dean of the Mechanical Engineering Faculty at TU-Liberec.
The results of the international research and training activities of the PI and a team of four UAB student-researchers during their visit to TU-Lodz and TU-Liberec were expected to provide new information on the mechanical properties and microarchitecture of nanoparticle bioceramic/biopolymer composites, and to broaden the scope of current program through the availability of additional facilities for materials fabrication, plasma processing, and characterization at host institutions. In particular, the IREE student-researchers planned to use extensively the modern SEM instrumentation at the host laboratories as this instrumentation in not readily available at UAB. Another expectation of this IREE trip was to initiate the long-term international collaboration with a purpose to advance the field of nanostructured, compositionally graded, and multiphase ceramic and polymeric materials for biomedical applications, as well as to train graduate and undergraduate students in physics of materials, nanoscience, and biomedical engineering technology.
The major criterion for the selection of the U.S. student-researchers was their involvement in the current project, demonstrated interest in learning new research techniques, obtaining international cultural and professional experience, and ability to travel abroad for an extended period of time. Two students already involved in the current project (C. Styres and A. Paulzak) were selected this way. One new undergraduate student from the UAB Science and Technology Honors (STH) Program was initially planned to be selected for this IREE. However, two highly qualified STH undergraduate students were identified by the STH Program (S. Sengupta and E. Ellis), and the STH program provided cost sharing that allowed a team of total four female undergraduate students to participate in this IREE. Each two students were assigned a project related to the current research program, and the foreign partner additionally involved the UAB students in their research activities.
The UAB student-researchers traveled from May 12 to August 6, 2008 and spent about 2 months at TU-Lodz, and 1 month at TU-Liberec. Dr. Stanishevsky traveled for two weeks starting simultaneously with student-researchers to help them at the initial stage of their 3-month program in the TU-Lodz and TU-Liberec, and to initiate broader collaboration.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

The research program of IREE participants included several focused research projects within the topics of current NSF award, namely, (i) fabrication of nanoHA/biopolymer composites with high wt.% loading of non-aggregated ceramic nanoparticles using gelatin as a representative material, (ii) studies of the microstructure and microarchitecture of the composites using SEM and TEM, (iii) comparative studies of viscoelastic properties using nanoindentation instrumentation at the partner's laboratories, and (iv) initial studies of plasma treatment on the properties of these composites.
The IREE student-researchers prepared a variety of samples of the nanoHA/polymer composites during 2007 Fall and 2008 Spring semesters before the IREE trip. Two new undergraduate students who were selected through the UAB STH Program (see above) student, in part, studied first the science behind the project topics and obtained pre-trip training mostly in Spring semester 2008 on the fabrication of nanoHA particles and composite materials, and mechanical properties analysis. All students practiced using needed analytical instrumentation at the foreign host laboratories in the progress of their work there (Fig.1a,b).

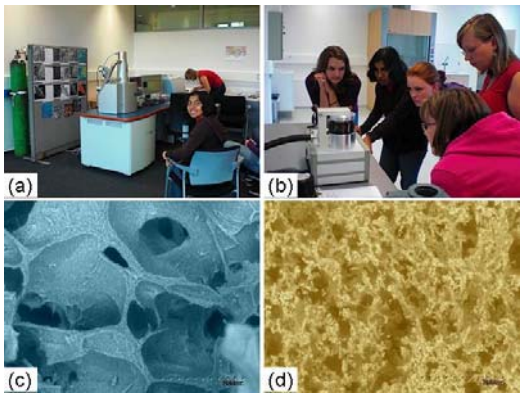

Figure 1. UAB students received training in SEM in TU-Lodz (a,b) and used SEM to study their samples of nanoHA / biopolymer composites (c,d).

The TU-Lodz partner, Dr. Mitura, was the major advisor to the IREE students and the coordinator of their activities both at TU-Lodz and TU-Liberec (jointly with Dr. Louda at the latter). He linked each UAB student to the mentor (either graduate student or junior researcher) in his group. The TU-Lodz and TU-

Liberec mentors were responsible for the UAB students' IREE and broader research/educational activities. The PI, Dr. Stanishevsky, helped UAB students to settle in Lodz and to initiate the program. He was then communicating with foreign partners and UAB students via e-mail and Skype conferencing on regular basis during the program.

The results of structural and morphological studies of fabricated nanoHA/gelatin composites with different wt.% ceramic nanoparticle loading have shown that the efficiency of nanoparticles loading depended strongly on the particle origin and surface modification. An approach has been proposed to prepare the nanoHA/gelatin composites with the uniform ceramic nanoparticle loading of 50 wt.% and above. The approach includes preparation of a solvent-free nanoHA/gelatin precursor using the surface-modified HA nanoparticles which provide uniform particle distribution in polymer, subsequent dissolution of the precursor in appropriate solvent followed by the electrospinning to fabricate the fibrous composite. The approach has been tested with bulk and freeze-drying procedures and up to 70 wt.% uniform particle loading without aggregation has been achieved. Fig.1c shows an SEM image of uniformly loaded porous 50 wt.% nanoHA/gelatin material, whereas Fig.1d shows substantial aggregation of HA particles. The testing of electrospun composites with high nanoparticle loading (at least 50 wt.%) is planned for the 3rd year of the main project.

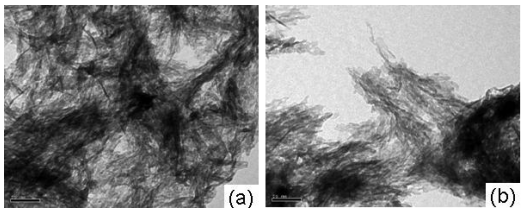

Figure 2. TEM studies in TU-Lodz have shown uniform nanoparticle distribution in (a) 70 wt% and (b) 50 wt.% nanoHA / biopolymer composites prepared in UAB.

The TEM studies confirmed that HA nanoparticles are indeed uniformly distributed and are not aggregated within the polymer matrix, as shown in Fig.2a,b.

The nanoindentation studies were conducted in parallel using similar MTS Nanoindenter G200 tools at UAB and TU-Lodz on several types of nanoHA/gelatin composite materials with various nanoHA/polymer ratios, including bulk, porous freeze-dried, porous electrospun, and pressure-consolidated composite constructs. The studies have demonstrated close results despite the variations in the measurement procedures. These results of microstructural and mechanical properties study will be presented, if the abstract accepted, at the MRS Fall 2008 Meeting in Boston [1].

One of the experiments proposed by the Polish partner included RF or glow-discharge plasma treatment of such composite materials to crosslink the biopolymer. This was thought to be and interesting approach as the data on the effect of plasma treatment of such composites on their cellular response and mechanical properties could not be found in literature to the best knowledge of the PI. The IREE student-researchers received training on the plasma equipment at TU-Lodz (Fig.3). They used both bulk porous and electrospun pure polymer and nanoHA/gelatin composites for rf-plasma processing in several mixtures of argon, hydrogen, and nitrogen in both TU-Lodz and TU-Liberec laboratories. Preliminary FTIR microscopy data of the plasma-treated bulk and electrospun samples the IREE students sent back to UAB have shown little chemical changes in the nanoHA/polymer composites, while the effect of cross-linking has been observed. It was concluded that rf-plasma processing can be a potentially an attractive way to cross-link polymers for biomedical applications. The TU-Liberec partner is expected to send one graduate student to UAB for 3 months in Fall 2008 to continue these investigations.

Figure 3. Graduate student Damian Batory from TU-Lodz instructs UAB students how to use rf-plasma equipment for the processing of materials. The insert shows a sample treated in rf-plasma.

BROADER IMPACTS OF THE INTERNATIONAL COOPERATION

During his 2-week trip in May, 2008, Dr. Stanishevsky has visited the Center of Excellence NANODIAM at the Technical University of Lodz, Poland, and the Center's partners at University of Life Sciences,

Warsaw, Poland, Technical University of Liberec, Czech Republic, Le Laboratoire de Matériaux (Materials Science Lab) de Ecole Catholique d'Arts et Métiers (ECAM), Lyon, France, and Ecole Nationale Supérieure d'Arts et Métiers (ENSAM), Cluny Center, France (Fig.4). Dr. Stanishevsky has given technical seminars at TU-Lodz (audience of ~50 graduate students, postdocs, and faculty, Fig. 4b), TU-Liberec (~30 graduate students), and ECAM (~70 undergraduate students). The current collaboration, the potential for the future research activities, and programs involving student exchanges have been discussed. For example, the potential for undergraduate engineering student exchange program was discussed in ECAM with Ms. Edith Frey, (ECAM Director of International Relations). This information was later sent by PI for the consideration by the UAB Study Abroad Program. One problem for the potential exchange program has been noted, namely, the big difference in the academic year schedules of the U.S. and European universities. Dr. Stanishevsky has also discussed further collaborative research activities with partners at TU-Liberec in the area of electrospinning processes, e.g. "Nanospider" process which is the next-generation electrospinning process in preparing nanofiber polymer and composite materials over the large areas (more that 1 meter wide, Fig.4c).

Figure 4. Dr. Stanishevsky has visited laboratories and gave seminars in five institutions, including (a,b) Technical University of Lodz, Poland, (c) Technical University of Liberec, Czech Republic, and (d) ENSAM, Cluny Center, France.

As the follow up of his IREE activities, Dr. Stanishevsky has been invited to present a talk at VaPSE International Conference in Hejnice, Czech Republic, in October, 2008. Also, during year 2007, following the submission and preparation stage of IREE proposal, Dr. Stanishevsky and Dr. Mitura (NANODIAM, TU-Lodz) have also discussed broader possibilities for collaboration and submitted a Materials World Network (MWN) proposal in the area of nanostructured carbons for biomedical applications to their corresponding funding agencies. Both partners have received funding for these MWN activities that will further strengthen and broaden their collaboration in the area of nanostructured biomedical materials research and applications. It is expected that this new NSF funded 3-year MWN project at UAB (PI: A. Stanishevsky, Co-PI: Y.Vohra) will support, in part, the international research and educational short and medium-term visits of 4 - 5 UAB students, junior researchers, and faculty annually to NANODIAM and its partner institutions. Similar number of researchers from NANODIAM is expected to visit UAB.

The team of four UAB IREE female student-researchers has had a multitude of broader activities both in Poland and in Czech Republic. The students were accommodated in the dormitories on campus at both destination sites, and communicated extensively with students from different European countries. During their almost 2-month stay in TU-Lodz (Dr. Mitura), the UAB students were additionally involved in several research projects in the area of nanomaterials, which extended their experiences beyond the scope of the original IREE program. All UAB students presented their finding at TU-Lodz at Dr.Mitura's group seminars. Two students produced sufficient results in these additional projects to be included in joint submissions of the research articles [2,3]. One planned research activity was not realized due to the scheduling conflicts, namely, a visit to Wroclaw University of Technology to analyze the samples using HRTEM in addition to regular TEM. The IREE students were constantly advised by their Polish peers on the interesting activities around, e.g., they enjoyed the 63rd Anniversary of TU-Lodz week of festivities, visited Warsaw, Gdansk and Baltic Sea shores. According to the responses from the Polish and Czech mentors (D. Batory, W. Kacharowski, A. Karczemska, P. Louda, and Z. Rozek), the collaborators in both those countries also learned much new information

Figure 5. (a) Dr. Burkhard Scholz from TECHNO-COAT, Zittau, Germany, tells the UAB students about the coating technologies of medical tools and implants; (b) UAB students at Saint-Gobain Advanced Ceramics, Turnov, Czech Rebublic. (Photos by A. Paulzak)

about the U.S. education and culture from the UAB IREE students.
During their stay in Liberec, Czech Republic, the IREE student-researchers have had a broad exposure to research and industrial establishments of different scale. Students were introduced to the "Nanospider" technology developed in TU-Liberec, which is the next-generation electrospinning process for the preparing of nanofiber polymer and polymer-based composite materials over the large areas. The TU-Liberec partner (Dr. Louda) organized their visits to Škoda Auto Factory and Research Laboratory (where students also studied their samples using SEM), small TECHNO-COAT Company (Zittau, Germany, Fig. 5a) specializing, in part, in functional coatings on medical tools and implants by various PVD techniques, the largest ceramics manufacturer in Central Europe, Saint-Gobain Advanced Ceramics in Turnov (Czech Rebublic, Fig.5b), where students learned about ceramics processing, such as nanoceramics made of 12-nm alumina particles, and NTC-New Technology-Research Center (NTC-Nové technologie - výzkumné centrum) in Plznen, which focuses in metallography, wear resistance, nanohardness and nanoscratch tests, and FESEM.

DISCUSSION AND SUMMARY

This IREE supplement to the current NSF award has clearly brought substantial benefits to the ongoing research program of the PI, and to the future international research and educational activities at the PI's institution.
The research activities completed during this IREE program helped to analyze the structure of various nanoHA/biopolymer composites, and to improve the knowledge base about their microarchitecture evolution in the dependence on the composite itself and composite's components fabrication methods. The experimental results obtained by the IREE student-researchers helped to design a new approach in achieving higher (up to 70 wt.%) nonaggregated nanoparticle loading into these composite materials, which is an important result for the further development and optimization of the nanofiber composite materials via the electrospinning technique.
Total four U.S. undergraduate female students were supported by this IREE program. Two of them were students from the new UAB Science and Technology Honors Program, which provided additional financial support. Besides their involvement in the research before the trip, all student-researchers obtained 3-month research training and cultural exposure in work and social environments in two countries (~2 months in Poland and ~1 month in Czech Republic).
The major broader impacts of this IREE program included the following: ***(i)*** Long-term collaborative activities between the TU-Lodz and UAB have been started within the new NSF-funded Materials World Network project in the field of nanostructured carbons (PI: Stanishevsky, Co-PI; Vohra), and ***(ii)*** New partnerships between the PI on the current NSF award and the scientists from ECAM (Lyon, France), ENSAM (Cluny, France), and TU-Liberec (Czech Republic) have been established, including new perspectives for broader participation of UAB students in the international research and student exchange programs.
Based on the PI and students observations, the following recommendations for the future implementations of the IREE program can be made:

- It would be good to clarify if shorter time visits of junior researchers and students, i.e., 1-3 week visits to run an important experiment or to make urgent measurements using the unique facilities in the foreign partner's institution are allowed within total 3 – 6 months of funded time spent in foreign institutions/laboratories for each researcher.
- In the IREE supplement proposals, there should be a requirement for the Plan "B" to be outlined for the cases if the equipment or project is not available to the expected extent for the IREE visitor in foreign institutions/laboratories during the visit, as well as for the case if the foreign colleagues/mentors have other unexpected duties that make them temporarily unavailable when the visitor has questions or needs help.

ACKNOWLEDGEMENTS

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BRIEF BIOGRAPHIES OF RESEARCHERS

Andrei Stanishevsky received his M.S. degree in Electrical Engineering from Belorussian State University of Informatics and Radiolectronics in 1985, and Ph.D. degree in Solid State Physics from the Institute of Solid State Physics & Semiconductors of the Belarus Academy of Sciences in 1996. Between 1985 and 1995 he worked as a junior and a senior researcher at the Belarus Academy of Sciences. Following a year of a post-doctoral training at the Pennsylvania State University in the Materials Research Laboratory in 1996-1997, he has been a research scientist at the University of Maryland, College Park. Since 2002, he has been an Assistant Professor of Physics at the University of Alabama at Birmingham, and an Associate Professor of Physics since October, 2008. Research interests include multifunctional inorganic nanoparticles and composite nanostructured materials for biomedical and optical applications.

Courtney Styres is a double Chemistry and Biology Major (senior). She is a Chemistry Scholar Fellow, a member of Sigma Alpha Lambda Honor Society and National Society of Collegiate Scholars. Since Fall 2006 Courtney works on ceramic nanoparticle-based composites with Dr. Stanishevsky under NSF award #CMS-0555778. She has also participated in UAB Physics NSF-funded REU Program for Summer 2007.

Sonda Sengupta is a Chemistry Major (sophomore) in UAB Science and Technology Honors (STH) Program. She has been selected by STH Program as a new undergraduate student to participate in this IREE in Summer 2008, and partially funded by STH program.

Erin Ellis is a Chemistry Major (sophomore) in UAB Science and Technology Honors (STH) Program. She has been selected by STH Program as a new undergraduate student to participate in this IREE in Summer 2008, and partially funded by STH program.

Audrey Paulzak is a Physics Major (senior) in UAB Physics Honors Program. She is Academic Achievement Chair in Alpha Xi Delta – Theta Phi. She received here first research experience in nanomaterials in Dr. Camata’s group in Spring 2007. Since Fall 2007 Audrey works on ceramic nanoparticle-based composites with Dr. Stanishevsky under NSF award #CMS-0555778.