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Mineral concentration dependent modulation of mechanical properties of bone-inspired bionanocomposite scaffold

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We demonstrate tunable mechanical properties of bone-inspired bionanocomposite scaffolds while maintaining the required viscoelasticity. Mechanical properties such as hardness and elastic modulus of the bionanocomposite scaffolds were controlled by varying mineral concentrations of the bioscaffold. In particular, higher calcium and oxygen contents in the bioscaffold resulted in a significant enhancement in hardness and modulus of the bionanocomposite. Moreover, the phosphorous content appeared to be a determining factor in the hardness and mechanical properties of the bionanocomposites. These results open up the possibility of designing new engineered biocompatible nanoscaffolds with desired and tunable biomimetic functions and biomechanical properties with significant potential for advanced bone tissue engineering platforms and bone substitutes. © *2011 American Institute of Physics*. [doi:10.1063/1.3607283]

As a specialized type of connective tissue, bone forms the skeleton structure of the body and is constructed at both the nano- and micro-levels, which controls its overall complex biomechanical properties. Mimicking bone structure presents an important frontier in the fields of nanotechnology, materials science, and bone tissue engineering, given the complex morphology of this tissue. There has been a growing interest in developing artificial bone-mimetic nanocomposite bioscaffolds with controllable mineral content, nanostructure and chemistry for bone, and cartilage tissue engineering and substitutes.[1–10] An ideal bonelike or bone-mimetic biomaterial would replicate the predominant co-alignment of the organic and mineral phases of the actual bone tissue architecture. This essentially involves nano- to micro-scale features of both the organization of collagen protein fibers in a characteristic three dimensional architecture and the integration of the important mineral constituent such as hydroxyapatite (HAP) nanocrystals within the collagen network.[11,12]

In addition to the ability to replicate mineral phases and structure of actual bone, understanding and controlling the mechanical properties (viscoelasticity, modulus, creep, and hardness) at the nano-, micro- and macro-scales of the synthetic composite bioscaffold materials are of immense importance in evaluating their medium and long term suitability at the submicron scale for various bone substitutes.[13–20] This is due to the fact that specific mechanical properties of such bone-regeneration nanoscaffolds are needed, since they have to withstand a high level of compression and torsion stresses after being implanted, which varies based on the location of implantation. Among other factors such as mineral filler size, shape, and distribution of filler in the matrix, changes in the mineral content can significantly influence the biomechanical functions of the scaffold.[13] For example, gradation in the mineral content in a composite bioscaffold has recently been shown to result in the variation of the scaffold stiffness (modulus) and which has been considered as an useful strategy for repairing the tendon-to-bone (soft tissue to hard tissue) insertion site via a tissue engineering approach.[19] This implies that controlling mineral composition in nanocomposite bioscaffolds which would allow simultaneous modulation and tailoring of the mechanical properties could potentially lead to flexible designs in engineered bioscaffolds for advanced bone tissue engineering and artificial bone substitutes that could be of use in the regeneration of tissues in various parts of the body.

Here, we report mineral-content-dependent mechanical properties of bone-inspired bionanocomposite scaffolds based on nanoindentation studies. We show the correlation of controlling calcium, oxygen, and phosphorous compositions and polymer-mineral interfaces, on the resulting changes in the viscoelastic and mechanical properties of bionanocomposite scaffolds.

Bionanocomposite scaffold materials with different mineral compositions that mimic the nanostructure of bone and bone chemistry were synthesized using a simple drop-casting method that has been reported earlier.[12] The drop-casting synthesis process allows integration of micro- and nano-scale binary features into nano-fibrous and porous biocompatible polymer scaffold structures along with compositional control of the mineral constituents that are required to match natural bone structures.[12] The concentration of HAP in the bionanocomposites was increased while lowering the concentration of $CaCO_3$ in order to reduce the Ca concentration. This was done to alter the mineralization level and the structural morphology of the bionanocomposite scaffold.

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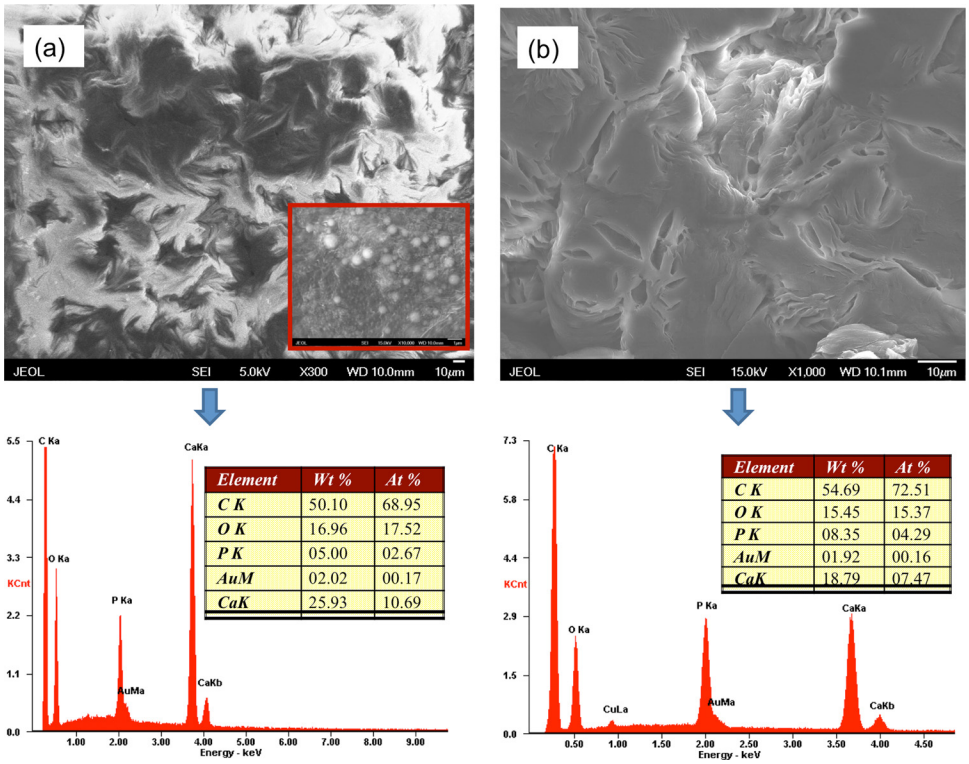

FIG. 1. (Color online) SEM overview images of (a) scaffold/porous structures of bionanocomposite consisting of polycaprolactone (PCL), hydroxyapatite (HAP), calcium carbonate ($CaCO_3$), collagen protein, and calcium and sodium based alginate and (b) surface morphology of bionanocomposite scaffold with higher HAP concentration. The inset of Figure 1(a) shows a higher magnified SEM image of HAP spheres embedded in a collagen rich fibrous matrix. The bottom panels show EDS analysis along with the corresponding elemental compositions that are shown in the table. EDS measurements show a calcium and oxygen rich bionanocomposite system with lower HAP concentration (a) and a phosphorous-rich bionanocomposite with higher HAP concentration (b). Note: the Au signal is due to the gold coating.

The scanning electron microscope (SEM) used for analysis of the bionanocomposites was a JEOL 7000 FE (resolution 1.2 nm at 30 KV) coupled with an energy dispersive spectroscopic (EDS) system for elemental analysis. Nanoindentation experiments to analyze the corresponding mechanical properties of the composites at the nanoscale were conducted for the bionanocomposite scaffolds. Indentation tests were conducted using a Hysitron TriboIndenter (Hysitron Inc., Eden Prairie, MN).[21,22] A Berkovich indenter was used, and 10 indents were applied to the samples to a maximum load of 30 μN with a 2 s hold time. The unloading curve (after the maximum indentation depth is reached) is used to calculate the reduced elastic modulus. The hardness is calculated based on the final indentation depth.

Figure 1 shows SEM images and the corresponding EDS data of bionanocomposite scaffold with different mineral concentrations. The SEM images show that these bionanocomposites have hierarchical surface roughness as well as micro- and nano-sized pores.

Figure 2 shows typical graphs of the load vs. indentation depth (h) for the bionanocomposite scaffold with different mineral concentrations as described above. The results show that the material is viscoelastic as evidenced by the creep at maximum load. The "creep-zone" (a region between the loading and unloading curves) was observed in both samples.

In these measurements, we used indentation depths ranging from 400 to 1000 nm as seen in Figure 2(b). In particular, when the overall morphology of the nanocomposites is controlled by the nanofiller-induced porosity and particle-polymer interfaces, indentation depths in excess of 1000 nm may be required for the accurate estimation of modulus, which was the case for the sample with higher HAP (and phosphorous).

Figure 3 shows plots (with standard deviation bars) of hardness and reduced modulus measured at different surface locations on the bionanocomposite scaffold surfaces prepared with higher calcium and oxygen and higher phosphorous. The plots indicate that both modulus and hardness values are more dependent on the measurement location for the bioscaffold sample prepared with higher HAP than the sample prepared with higher calcium and oxygen (greater surface roughness).

The inset of Figure 1(a) indicates HAP spheres of approximately 100–500 nm embedded within the nanofibers (~100 nm) of collagen that are organized parallel to each

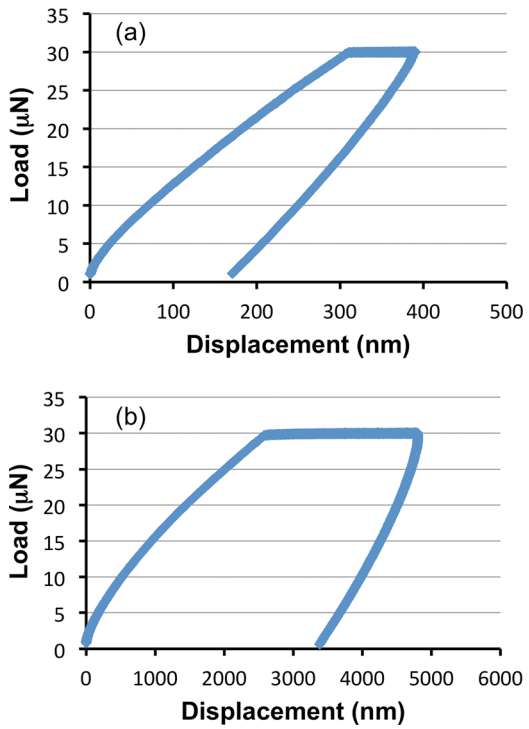

FIG. 2. (Color online) Typical graphs of the load vs. indentation depth for the bionanocomposite scaffold in the case of (a) higher calcium mineralized and (b) higher phosphorous (and HAP) mineralized.

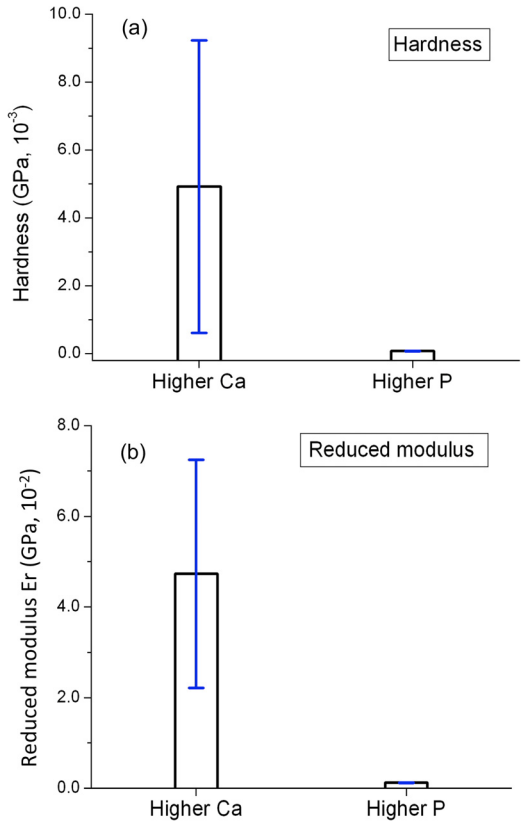

FIG. 3. (Color online) Variations in the hardness (a) and reduced modulus (b) of the nananocomposite scaffolds prepared with higher calcium (Ca) and higher phosphorous (P).

other, which is a crucial morphological arrangement for enhanced mechanical properties and which is normally found in healthy bone tissues.[11,20] Further details of the structural morphology of such bonelike bionanocomposite scaffolds have been reported elsewhere.[12] The SEM analysis shown in Figure 1(b) indicates a more uniform mixing of HAP granules and their arrangements within the bulk in this case. The EDS data with higher HAP concentration shown in the bottom panel of Figure 1(b) suggest a higher phosphorous to calcium ratio compared to the bioscaffold with lower HAP concentration (Figure 1(a)). It is therefore apparent that the higher HAP concentration raised the phosphorous level in the bioscaffold.

The results presented in Figure 3 suggest that the increased calcium and oxygen and decreased phosphorous contents increase the hardness and modulus values while still maintaining viscoelastic properties. However, sensitivity of these values to measurement location also increases, indicating possible formation of different polymer-filler near-surface morphologies, especially when phosphorous content in the nanocomposite has declined. In general, lower reduced modulus is obtained for the highly porous nanocomposite bioscaffold with higher phosphorous (and HAP) concentration (Figure 3(b)). This is consistent with the previously observed differences between moduli of HAP-collagen non-porous nanocomposite films and electrospun (highly fibrous) HAP-collagen nanocomposites.[23] It is noteworthy that an increase in Ca content by approximately 7% in the bionanocomposite scaffold resulted in more than a 3-fold increase in the modulus (Figure 3(b)).

Also, we observed that the hardness values of the bionanocomposite scaffolds can change by several orders of magnitude as a function of the HAP and Ca mineral content, which drastically influence surface roughness. A variation in the hardness of nearly three orders of magnitude was observed for the bioscaffold prepared with higher Ca (Figure 3(a)). Such large variations in mechanical properties in the bionanocomposite scaffold can be attributed to a number of factors. The most important is the formation of nanoparticle aggregates in random regions within the bionanocomposite leading to a hardening behavior, such as the case with iron carbides in alloy steels.

Aggregate formation also leads to large variations in modulus and hardness measurements at different locations on the same sample.[24] Depending on the biopolymer matrix viscoelastic behavior, this dependence on HAP nanoparticle loadings can vary significantly. In fact, this type of spatially dependent mechanical behavior is also observed in bone.

In conclusion, tuning the mechanical properties such as hardness and modulus of bone-like bionanocomposite scaffolds, by changing bone mineral concentrations while maintaining viscoelastic properties, demonstrates a framework for bone scaffold design and development. The results show that higher calcium and oxygen contents in the bioscaffold can dramatically enhance hardness and modulus. Our results suggest that a decrease in phosphorous may play a determining role in the mechanical properties of the bioscaffold. The approach of changing calcium and phosphorous, along with oxygen concentrations in the bioscaffold, can serve to tailor the mechanical properties and demonstrates the possibility of engineering new bioscaffolds to more closely match those of natural bone for advanced tissue engineering or bone substitute applications.

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