SUPPORTING INFORMATION

Spiral Layer-by-Layer Micro-Nanostructured Scaffolds for Bone Tissue Engineering

Ohan S. Manoukian
Aja Aravamudhan
Paul Lee
Michael Arul
Xiaojun Yu
Swetha Rudraiah
Sangamesh G. Kumbar

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1. Supplementary Texts and Results

In order to test the effectiveness of the LBL technique for HA deposition, an Alizarin red staining method was employed to identify successful calcium deposition. HA was uniformly deposited on the surface of the spiral scaffolds, as evidenced by the alizarin red staining assay (**Figure S2**). Increasing number of HA layers corroborated with increased alizarin red staining. Qualitatively, this is indicative of the amount of calcium loaded onto the scaffold with an increase in the number of HA bilayers. **Figure S2A** depicts minimal staining with alizarin red when no bilayers were deposited. In contrast, **Figure S2C and S2D** show substantial dark red staining, when 3 and 5 layers of HA were deposited on the spiral scaffolds, respectively. This change in color is indicative of a sizeable increase in calcium deposition, which is in agreement with theory as the number of bilayers of HA increases.

The deposition of HA onto the scaffold surface was confirmed using SEM and EDX spectroscopy. SEM images of the spiral scaffold with 5 layers of HA deposited without and with nanofibers are shown in **Figure S3A/B and 6C,** respectively. The formation of the HA and polymer layers on the substrates of the nanofiber coated spiral scaffolds can be observed. EDX analysis also confirmed the deposition of HA, measuring the presence of calcium and phosphorus on the surface of the scaffolds (**Figure S4**), while mineralization was not detected in scaffolds without layer-by-layer HA coating on the surface (data not shown). The Ca/P ratio of HA-coated scaffolds corresponded to that of the HA powder alone, indicating the success of the layer-by-layer coating process and revealing no adverse chemical interactions altering the structure of HA using the process.

2. Supplementary Figures

Figure S1: Schematic of testing depicting the various compositions of spiral scaffolds using zero, one, three, and five bilayers of HA with and without nanofibers. Cylindrical scaffolds without HA, with adsorbed HA, and with one, three, and five bilayers of HA were used as experimental controls.

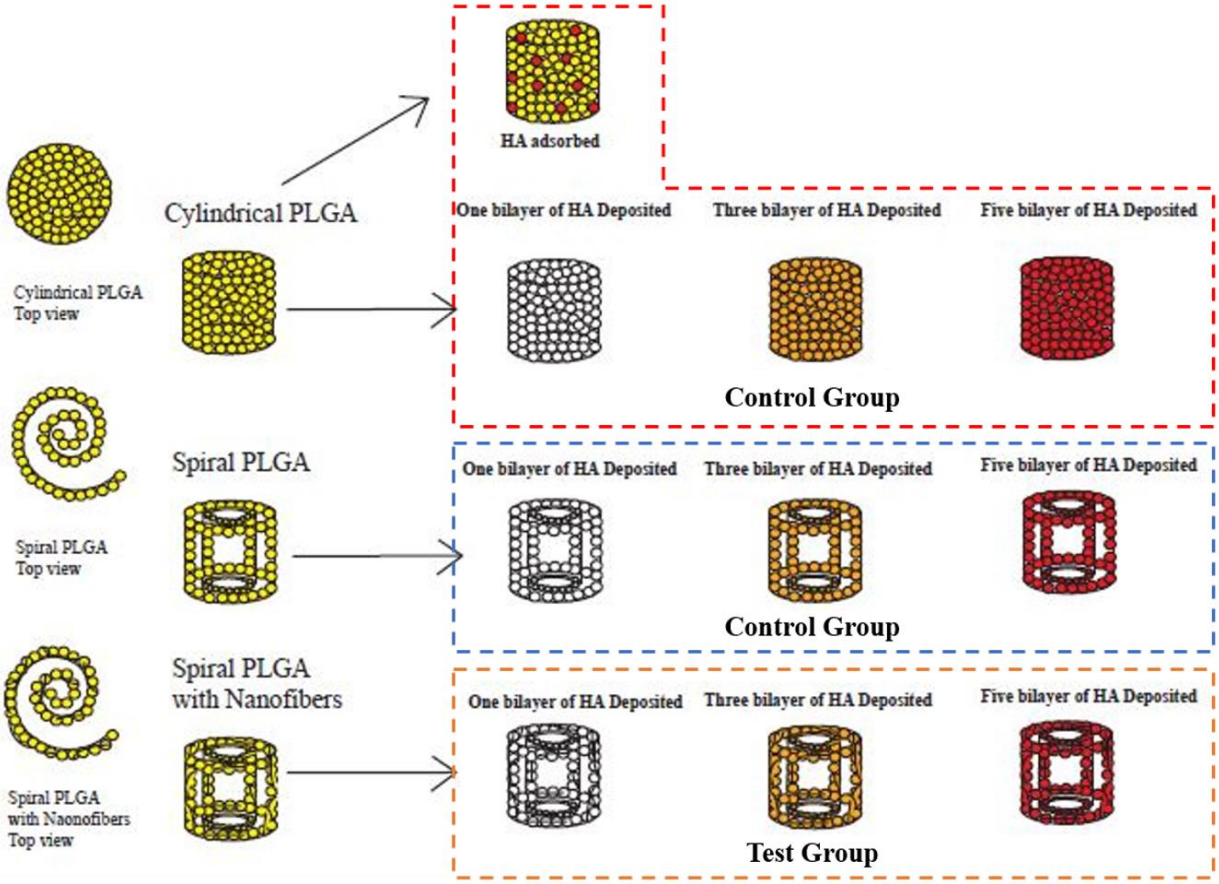

Figure S2: Layer-by-layer deposition of HA on nanofibrous spiral scaffold where Alizarin red assay shows the effect of number of HA bilayers deposited on scaffold. (A) control adsorbed HA, (B) one layer HA, (C) three layers HA, and (D) five layers HA.

Figure S3: SEM of 5 bilayers of HA deposited using the described layer-by-layer technique on spiral scaffolds (A) without nanofibers, low magnification; (B) without nanofibers, high magnification; and (C) with nanofibers.

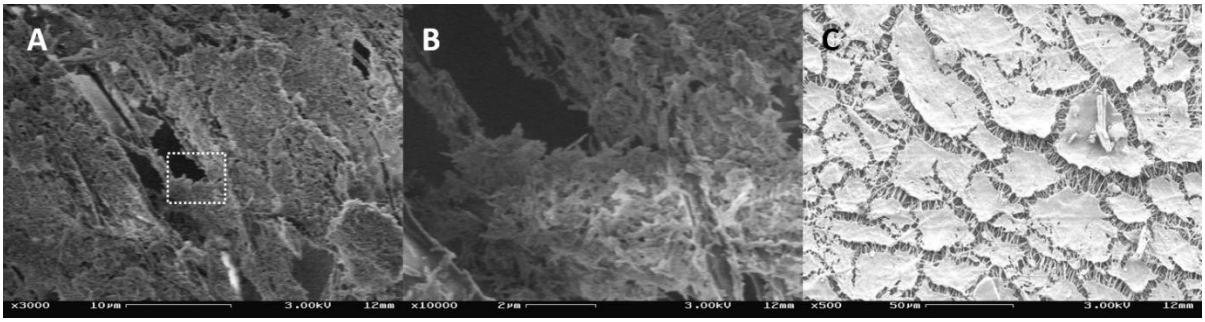

Figure S4: EDX spectra of HA-coated spiral scaffolds confirming strong presence of Calcium and Phosphorus ions.

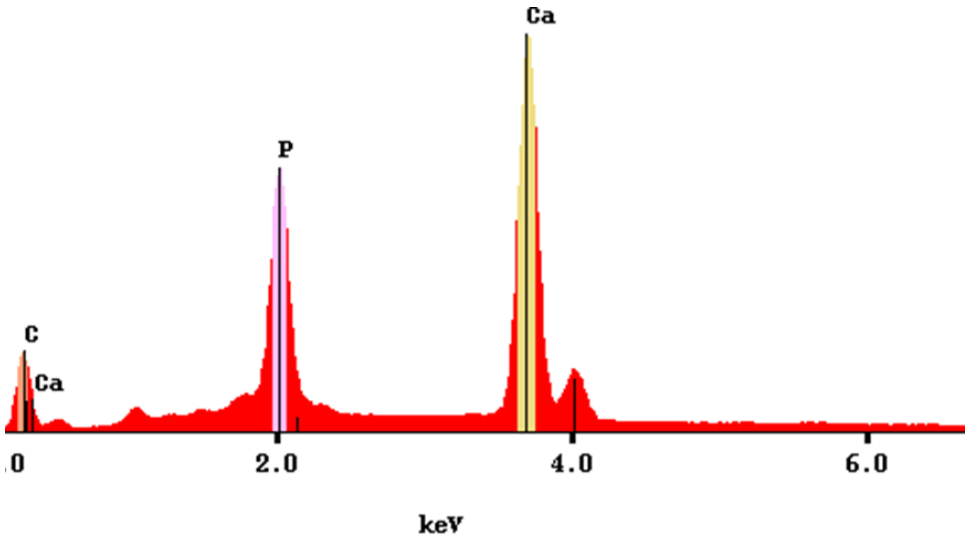

Figure S5: Full MTS study results showing all six tested groups including raw spiral scaffolds, spiral/HA scaffolds, spiral/fibers scaffolds, spiral/fibers/HA scaffolds, cylindrical scaffolds, and cylindrical/HA scaffolds. * Indicates significant increase in cell attachment as compared to cylindrical scaffolds, ** indicates significant increase in cell proliferation at day 3 as compared to cylindrical scaffolds with or without HA, # indicates significant increase in cell proliferation as compared to all scaffolds at days 7 and 14.

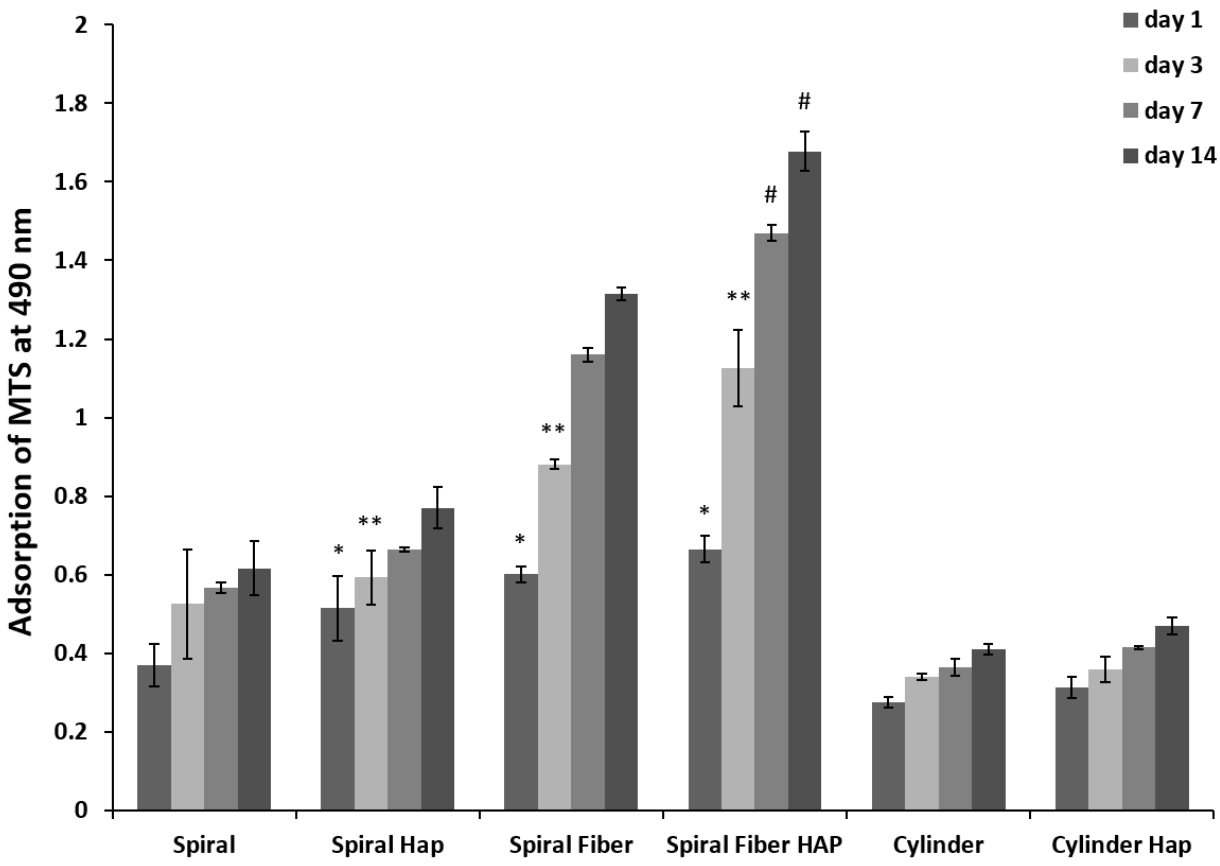

Figure S6: Full ALP study results showing all six tested groups including raw spiral scaffolds, spiral/HA scaffolds, spiral/fibers scaffolds, spiral/fibers/HA scaffolds, cylindrical scaffolds, and cylindrical/HA scaffolds. * Indicates significant increase in ALP activity as compared to cylindrical scaffolds coated with HA. ** indicates significant increase in ALP activity at day 7 as compared to all other scaffolds with or without HA, # indicates significant increase in ALP activity as compared to all scaffolds at days 14. $ indicates significant increase in ALP activity on cylindrical scaffolds coated with HA as compared to cylindrical scaffolds without HA on day 14.

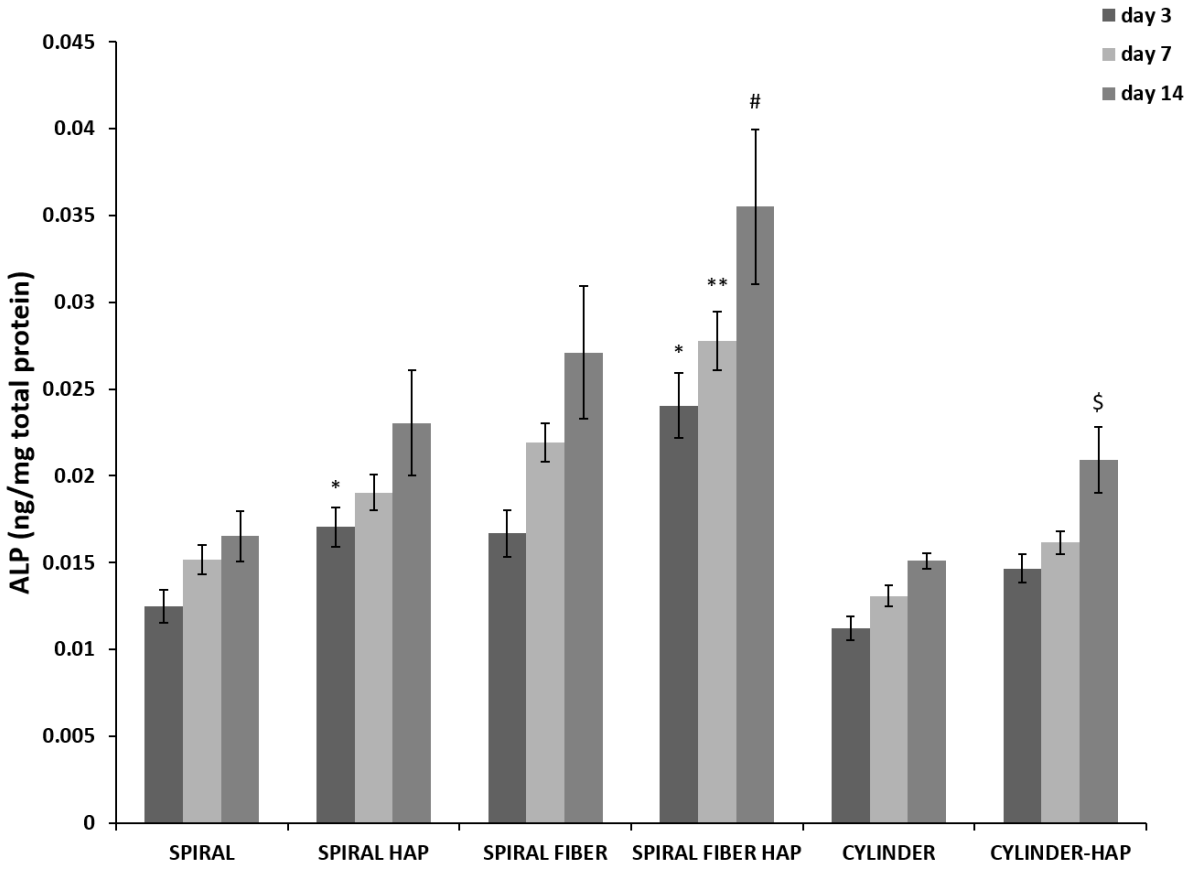

Figure S7: Full alizarin red calcium deposition study results showing all six tested groups including raw spiral scaffolds, spiral/HA scaffolds, spiral/fibers scaffolds, spiral/fibers/HA scaffolds, cylindrical scaffolds, and cylindrical/HA scaffolds. * Indicates significant increase in matrix mineralization as compared to cylindrical scaffolds coated with HA. ** indicates significant increase in calcium deposition at day 7 as compared to all other scaffolds with or without HA, # indicates significant increase in Calcium deposition as compared to all scaffolds at days 14. $ indicates significant increase in Calcium deposition on cylindrical scaffolds coated with HA as compared to cylindrical scaffolds without HA on day 14.