Supporting Information

Polycaprolactone coated 3D printed tricalcium phosphate scaffolds: *In vitro* alendronate release behavior and local delivery effect on *in vivo* osteogenesis

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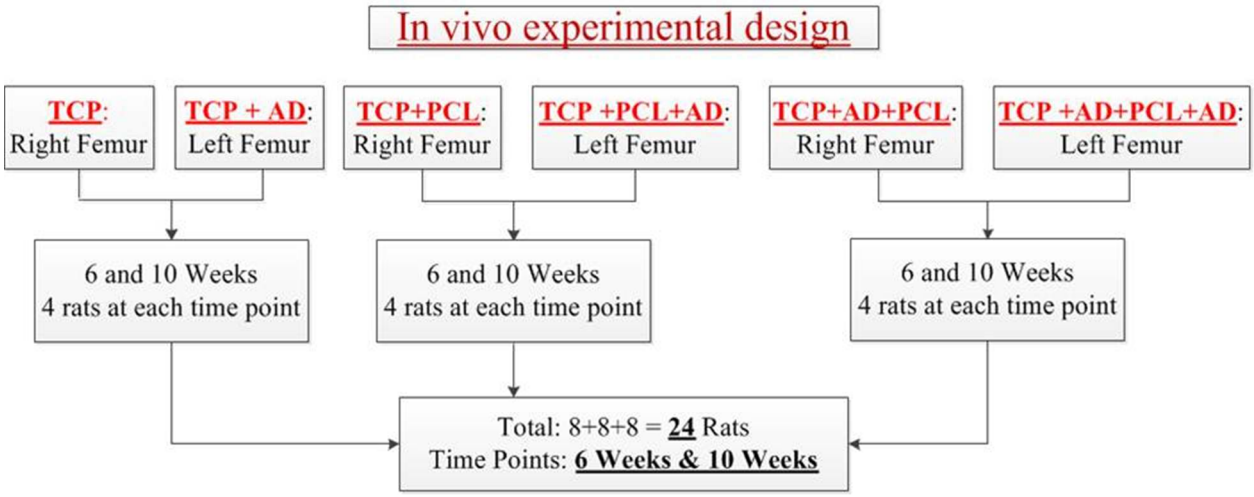

Figure S1. *In vivo* experimental design.

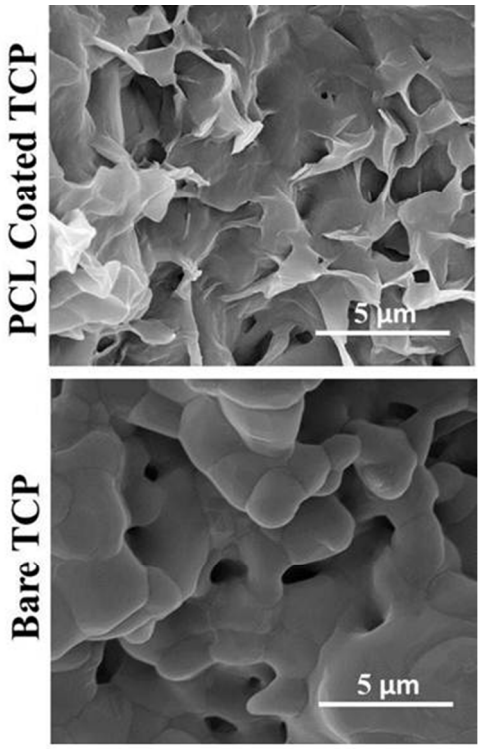

Figure S2. Surface morphology of the 3D printed bare TCP, and PCL coated 3D printed TCP scaffolds showing PCL coating made the TCP surface more roughened through the flake like PCL coating.

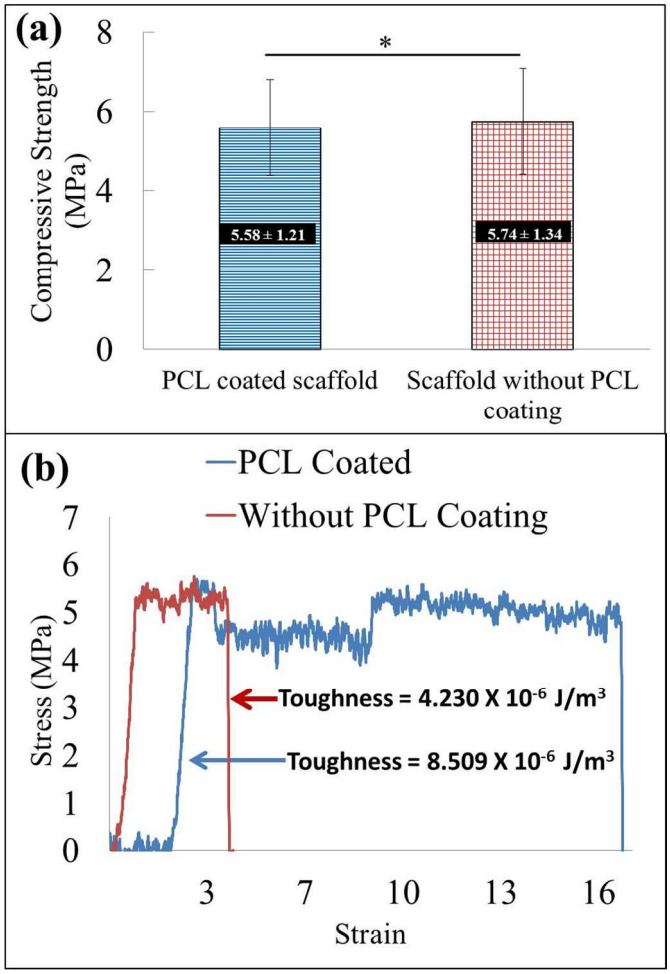

Figure S3. (a) Compressive strength comparison between PCL coated and non-PCL coated 3DP pure TCP scaffolds (* $p > 0.05$, n=6); (b) Stress vs strain plot showing the toughness comparison between PCL coated and non-PCL coated 3DP pure TCP scaffolds.

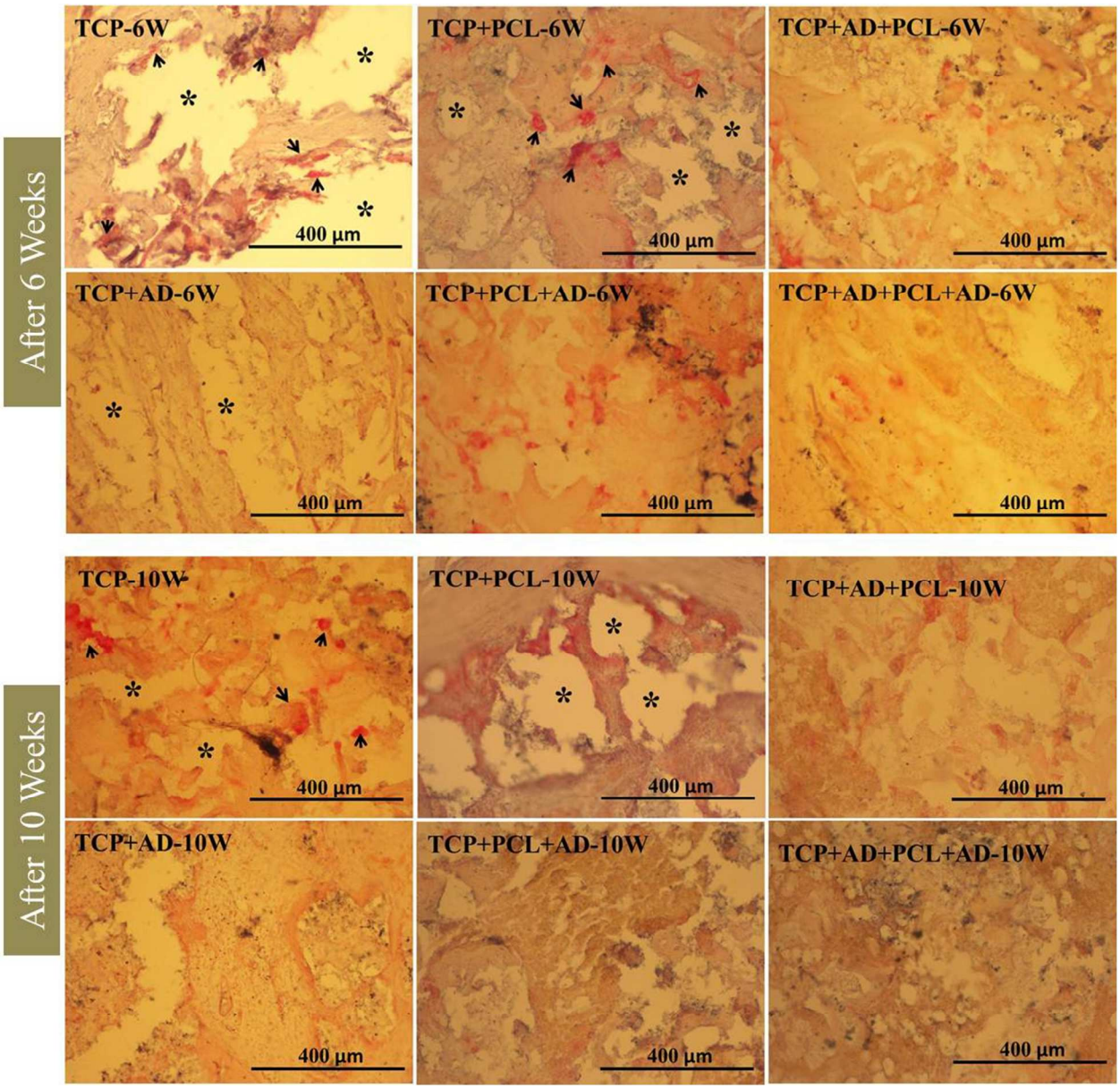

Figure S4. Photomicrographs showing TRAP positive cells activity after 6 and 10 weeks. Arrow heads indicate TRAP positive staining: Red; Star (*) marks indicate acellular regions derive from scaffold; BM = Bone marrow.

Experimental and Methods

In vitro cell material interactions

In vitro bone cell materials interactions on six different scaffold compositions namely, (i) TCP (bare TCP), (ii) TCP+AD (AD loaded TCP), (iii) TCP+PCL (PCL coated TCP), (iv) TCP+PCL+AD, (v) TCP+AD+PCL and (vi) TCP+AD+PCL+AD were investigated using human fetal osteoblast cells (hFOB) for 3, 7 and 11 days of incubation period. Cells used in this study were immortalized osteoblastic cell line derived from human bone tissue. All scaffolds were sterilized by autoclaving at 121°C for 30 min prior to PCL and AD coating. AD and PCL coating were carried out under a sterilized hood, and left overnight for drying in the same sterilized environment before cell culture. Aliquot of 50 µL cell suspension containing 2×10^5 cells were seeded directly on each sample placed in the wells of 24-well plates. Cells were incubated at 37 °C under 5 % CO2 / 95 % humidified air atmosphere for 30 minutes for better cell adhesion. After that, a 1 mL aliquot of DMEM media enriched with 10% fetal bovine serum was added to the surrounding of each sample in the well and returned to the incubator. The culture media was changed every alternate day for the duration of the experiment.

Cell morphology

Cell morphology was assessed by SEM observation. For this, scaffolds were fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer overnight at 4 °C. Post fixation was performed using 2% osmium tetroxide (OsO4) for 2 h at room temperature. OsO4 fixed samples were then dehydrated in an ethanol series (three times for each of 30%, 50%, 70%, 95%, and 100%), followed by a hexamethyldisilane (HMDS) drying procedure. Dried samples were then gold coated (Technics Hummer V, San Jose, CA, USA), and observed under an FESEM (Everhart-Thornley Detector).

Cell proliferation using MTT assay

The MTT assay (Sigma, ST. Louis, MO) was performed for 3, 7 and 11 days of incubation to assess hFOB cell proliferation on the scaffolds. MTT solution (5 mg mL^{-1}) was prepared by dissolving MTT in PBS, and filter sterilized. 100 µL diluted MTT solution was added to each scaffold in 24-well plates followed by addition of 900 µL Dulbecco's Minimum Essential (DME) medium. After 2 h of incubation at 34 °C, scaffolds were taken into new well plate and 1 mL solubilizer (made up of 10% Triton X-100, 0.1 N HCl, and isopropanol) was added to each well to dissolve the formazen crystal. 100 µL of the resulting supernatant was transferred into a 96-well plate, and read by a Biotek Synergy 2 SLFPTAD microplate reader (Bioteck, Winooski, VT, USA) at 570 nm. Three biological and three technical replicates were used for each scaffold composition.

Results

In vitro cell morphology & cell proliferation

SEM micrographs of hFOB cells showing cell adhesion and proliferation on the scaffolds after 3, 7 and 11days of culture are presented in **Figure S5**. All scaffolds showed good cell adherence, proliferation and cell ingrowth into the pores suggesting that AD and PCL coating did not cause any deleterious effect on hFOB cells. hFOB cell proliferation on these scaffolds was studied using MTT assay. **Figure S6** presents the cell densities observed on the scaffold compositions as a function of culture time. Only bare TCP showed a significantly higher cell density than any other compositions after 3-day culture period. With the increasing culture time, cell density was increased on all compositions after 7-day culture except TCP+PCL+AD, where cell density remained almost unchanged. After 7 and 11-day culture periods, there was no

significant difference (*$p > 0.05$) in cell density among TCP, TCP+PCL, and TCP+AD+PCL. There was a significant difference between these three and TCP+PCL+AD. Cell density assessment from PCL coated TCP scaffolds (TCP+PCL) demonstrated that PCL coating did not cause any hindrance on cellular adhesion and proliferation on the scaffolds with PCL coating. **Figure S6a and b** shows hFOB cells with many filamentous extensions, nice cell attachments and spreading on the scaffolds with PCL coating.

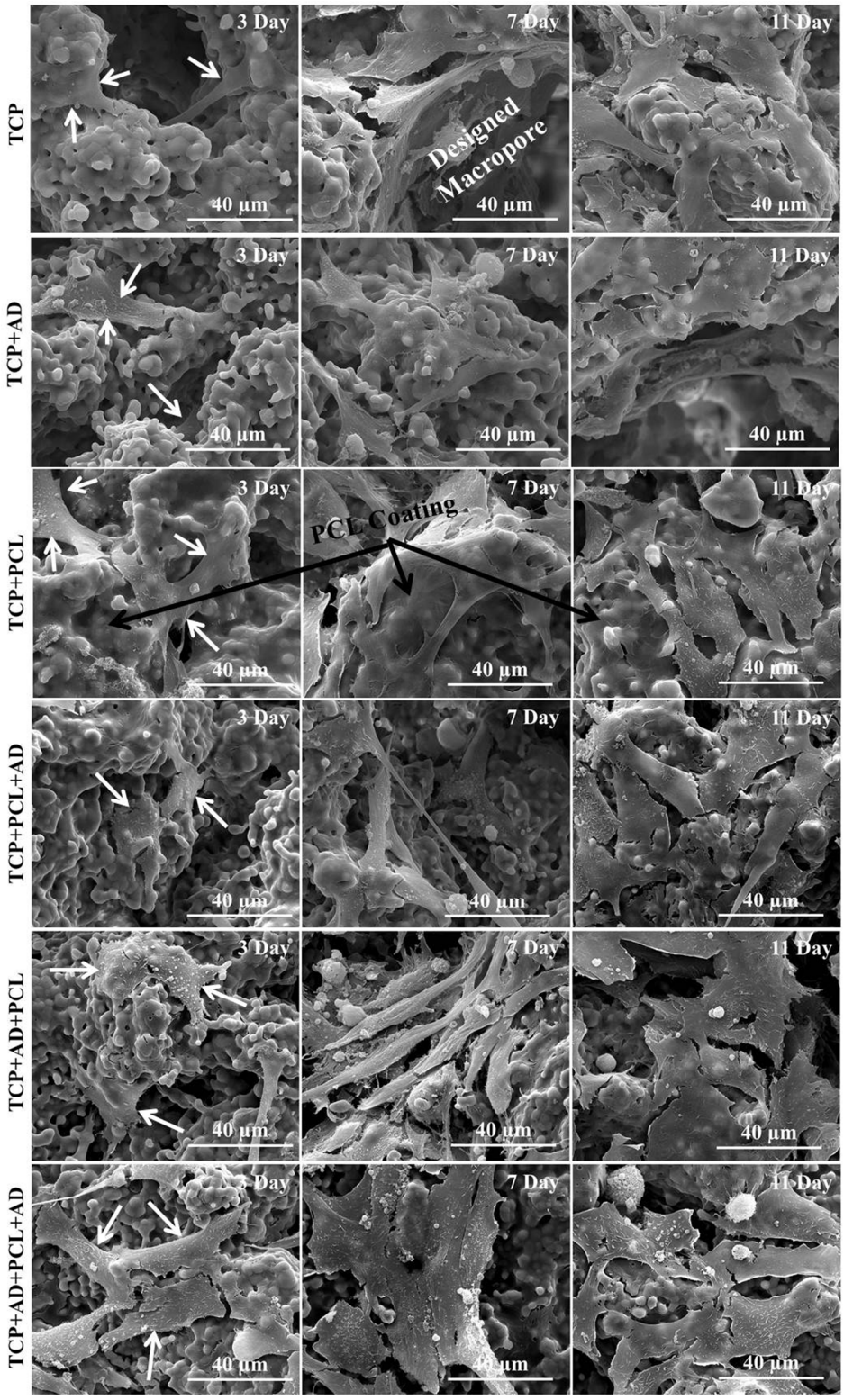

Figure S5. SEM micrographs of hFOB cells showing the cell adhesion and proliferation on the scaffolds after 3, 7 and 11days of culture. White arrows indicate cells.

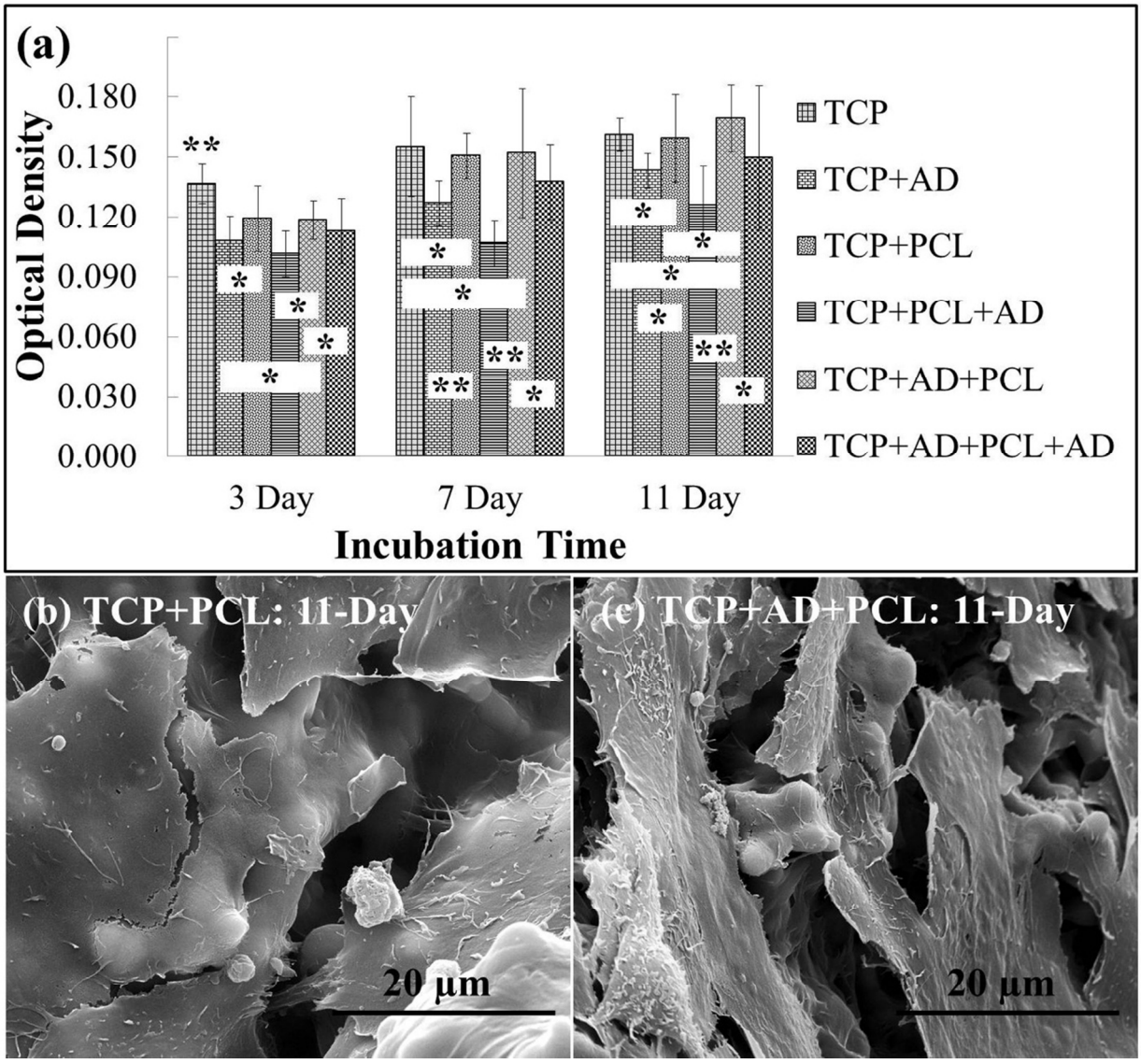

Figure S6. (a) MTT assay of hFOB cells on the PCL and/or AD coated 3D printed scaffolds after 3, 7, and 11 days (**$p < 0.05$, *$p > 0.05$, n = 3); higher magnification SEM images showing good cell adhesion and proliferation on the TCP+PCL (b) and TCP+AD+PCL (c) coated 3DP scaffolds.