2.3. Electrospinning (e-spinning) of PCL nanofibrous matrix and immobilization of silk protein fibroin through aminolysis:

The PCL matrices (2 cm × 2 cm) were submerged in absolute alcohol-water mix (1:1 v/v) for 2-3 h, washed with copious amounts of deionized water, and subsequently immersed for an hour in a 10 wt% solution of 1, 6- hexanediamine in 2-propanol solution at 37 °C. Over the next 24 h, the matrices were rinsed in deionized water, at room temperature. This was followed by 24 h of vacuum drying at 30 °C to remove any free 1, 6-hexanediamine. The aminolyzed matrices were immersed for 3h, in a room temperature solution of 1% (w/v) glutaraldehyde (GA). The matrices were rinsed using deionized water 8-10 times to remove any free GA. The matrices were then put for 24 h in 2 wt% silk fibroin protein solution and maintained at 2-4 °C, followed 24 h in deionized water to rinse off excess SF.

2.12.2. Cell viability (MTT) and proliferation (alamar blue) assay:

The matrices were incubated in 5 mg/ml MTT stock solution, diluted at 1:10 ratio with PBS (pH 7.4). Formazan crystal formed in this incubation was dissolved in dimethyl sulfoxide. Absorbance of these solutions was measured, following manufacturer's protocol, in a spectrophotometer (Bio-Rad, iMark).

Alamar blue dye was diluted in the culture media, at a ratio of 1:10 and scaffolds were incubated in this solution for 4 h, in the dark. Dye reduction extent was determined using spectrophotometry at absorbance wavelengths of 570 and 600 nm, in a microplate reader (Thermo Scientific Multiskan Spectrum, Japan). The percentage of dye reduction was calculated from the following equation:

$$\% \text{ AB reduction} = [(\varepsilon_{ox}\lambda_2)(A\lambda_1) - (\varepsilon_{ox}\lambda_1)(A\lambda_2) / (\varepsilon_{red}\lambda_1)(A'\lambda_2) - (\varepsilon_{red}\lambda_2)(A'\lambda_1)] \times 100$$

In Equation 5, $\varepsilon\lambda_1$ and $\varepsilon\lambda_2$ represent molar extinction coefficients for alamar blue at 570 and 600 nm respectively, ε_{ox} in oxidized and ε_{red} in reduced form; $A\lambda_1$ and $A\lambda_2$ were absorbance of the test wells; and $A'\lambda_1$ and $A'\lambda_2$ were the absorbance of the negative control wells. All given pairs are values at 570 and 600 nm.

2.13.5. Total protein analysis:

At predetermined time-points, samples were washed with PBS and lysed for 30 min on ice using the lysis buffer. A set of dilutions of the standard solution (0–2000 µg/mL) was used to make the albumin standard (BSA) curve. Protocol given in the kit was used to prepare the working reagent. At a 1:8 (v/v) ratio, sample (25µL) was mixed with reagent (200µL), 37 °C, for 30 min. Absorbance of the mix at 562 nm was measured in a Microplate Reader (Thermo Scientific Multiskan Spectrum, Japan). Protein content of the scaffolds was measured before seeding to find the net protein produced by the seeded osteoblasts.

2.13.7. Cell viability, Live/dead assay

40 nM calcein AM and 20 nM ethidiumhomodimer were used to preapare a dye solution in DMEM, without FBS. Cell laden matrices after 5 days of culture were collected and rinsed thrice with 1x PBS. Scaffolds were stained by 30 min immersion in the dye solution, in the dark. Following staining, the matrices were cleansed with PBS and examined through confocal microscopy (FV 1000 Advance software v. 4.1, Olympus), at excitation of 488 nm

and 543 nm lasers. Live cells detected had stained green from calcein and dead cells stained red from ethidiumhomodimer.

2.13.12. Calcium assay:

The scaffolds were rinsed two times in PBS and homogenized with 0.6(N) HCl. Continuous shaking for 4 h was used at 41°C to extract calcium. This lysate was centrifuged for 5 min at 1000g. Supernatant thus yielded was used for calcium quantification. Calcium content of the nanofibrous scaffolds had been measured prior to cell seeding. This value was subtracted from the calcium content of the cell laden scaffolds to obtain the net calcium deposited. Spectrophotometry with cresolphthalein complexone (Sigma) was used to determine calcium concentration in lysates. Reagents were added to the lysate and kept for 3 min before reading absorbance of the samples at 575 nm in a microplate reader. A standard curve of absorbance had been generated using serial dilutions of the standard calcium solution provided by Sigma. This curve was used to convert absorbance values to a quantitative value of calcium content.

Figure supplement 1: Weight gain of the non-mulberry silk fibroin grafted PCL nanofibrous matrix (NSF-PCL) after grafting of 4-META as a function of reaction time.

Figure supplement 2: (a) Water uptake (%) of NSF-PCL, NSF-PCL/3Vand NSF-PCL/5V composite nanofibrous matrices in double distilled water at definite time intervals. The addition of nHAp enhanced the swelling ability of the nanofibrous matrices. All the matrices finally reached equilibrium after 4 h.(b) The elution of Ca^{2+} ion from the different nHAp deposited NSF-PCL nanofibrous matrices to ultra-pure water against the soaking times.(c) The degradation of composite nanofibrous matrices at 37 °C and 100 rpm: proteinase K (Tritirachium album) solution and (d) buffer solution (PBS, pH 7.4), which served as control. The extent of degradation is expressed in terms of weight remaining at each time point. n=3 at each time point.

Figure supplement 3: The response of osteoblast like cells (MG-63) seeded on the nHAp deposited composite nanofibrous matrices and cultured for 14 days at 37^{o}C and 5% CO_2 humidified atmosphere.(a) Viability and (c) proliferation of the cells during culture for 14 days, indicating superior cell response on NSF-PCL/3V and NSF-PCL/5V than control (NSF-PCL). NSF-PCL/5V showed maximum cell viability and proliferation. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$, n=3 at each time point (One way ANOVA followed by Tukey's Honest Significant difference test).

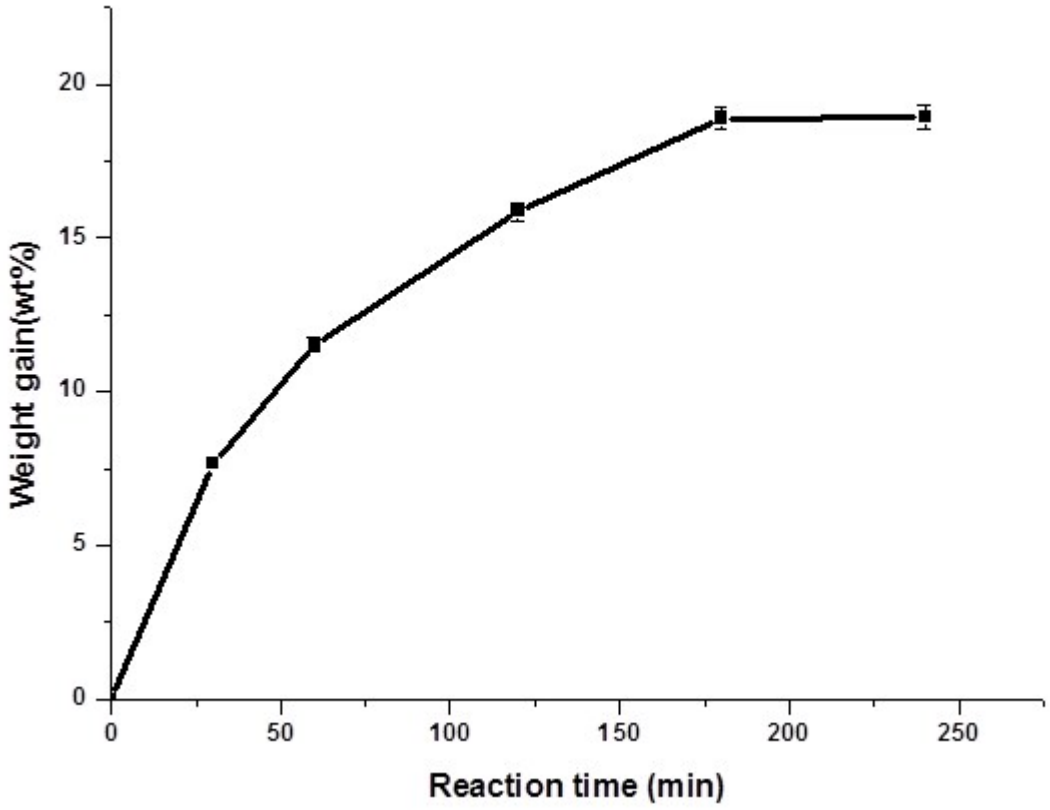
Weight gain(wt%)
Reaction time (min)
0
5
10
15
20
0
50
100
150
200
250

Fig. S1

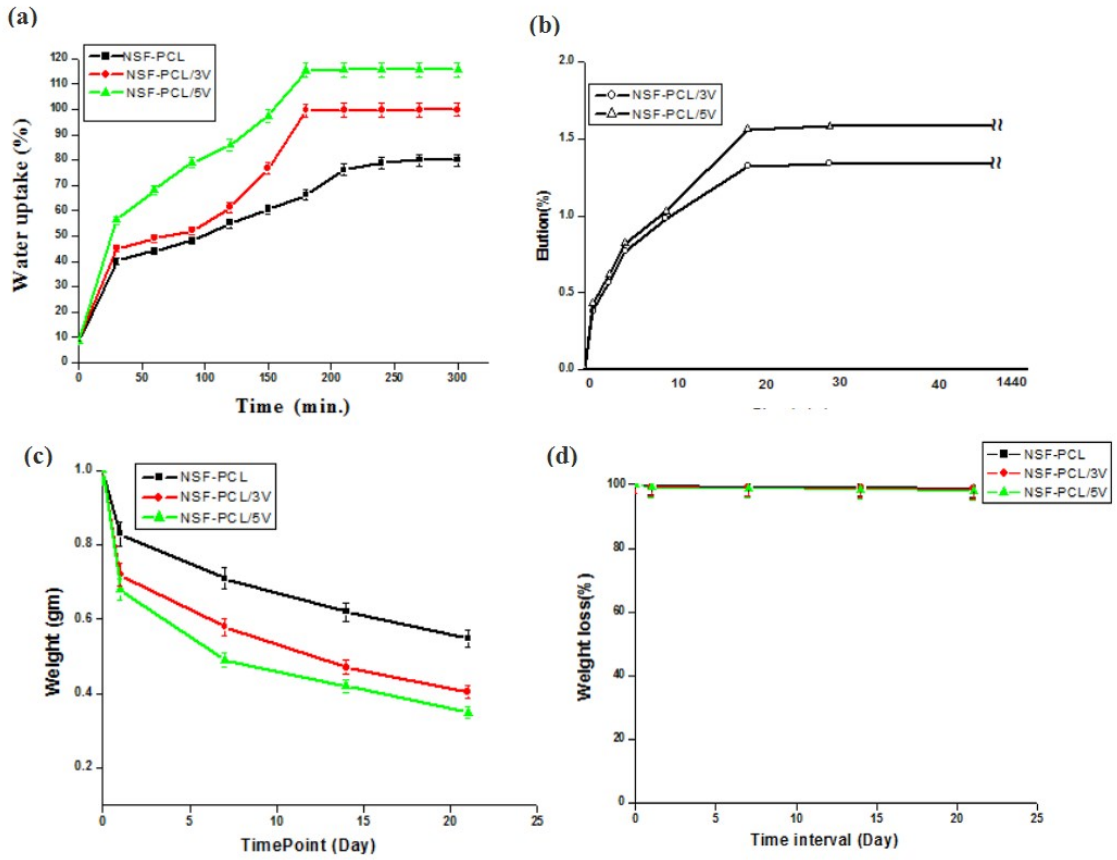
(a)
NSF-PCL
NSF-PCL/3V
NSF-PCL/5V
Water uptake (%)
Time (min.)
(b)
NSF-PCL/3V
NSF-PCL/5V
Elution(%)
(c)
NSF-PCL
NSF-PCL/3V
NSF-PCL/5V
Weight (gm)
TimePoint (Day)
(d)
NSF-PCL
NSF-PCL/3V
NSF-PCL/5V
Weight loss(%)
Time interval (Day)

Fig. S2

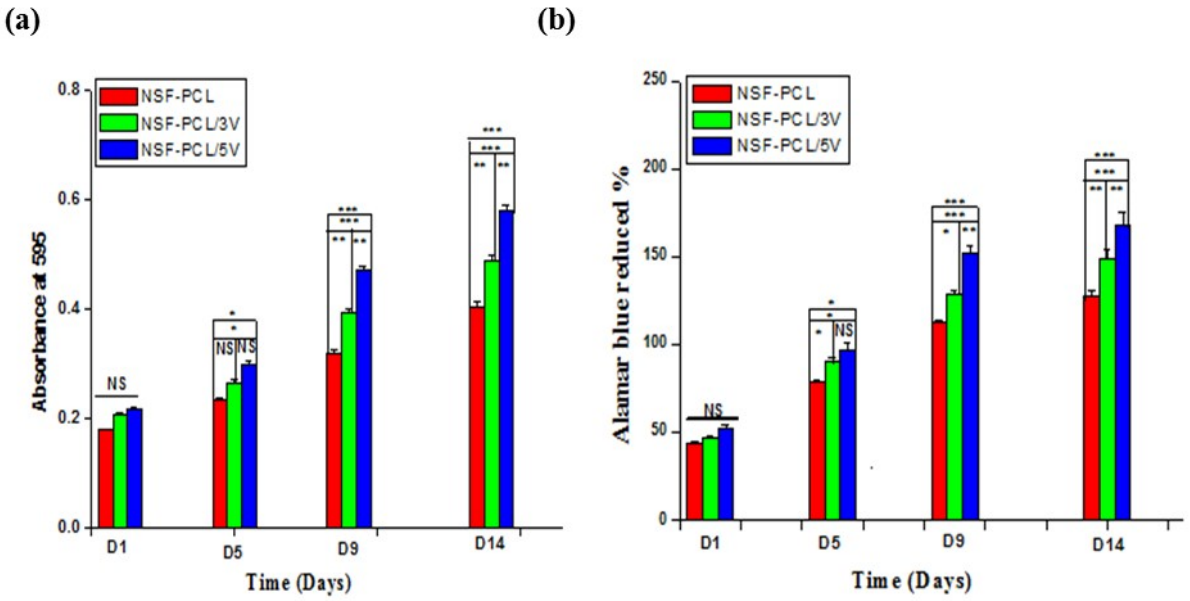
(a)
(b)
NSF-PCL
NSF-PCL/3V
NSF-PCL/5V
Absorbance at 595
Alamar blue reduced %
Time (Days)
D1
D5
D9
D14
NS

Fig. S3