Exploring the potential of aloe vera and honey extracts loaded bi-layered nanofibrous scaffold of PCL-Col and PCL-SBMA mimicking the skin architecture for the treatment of diabetic wounds

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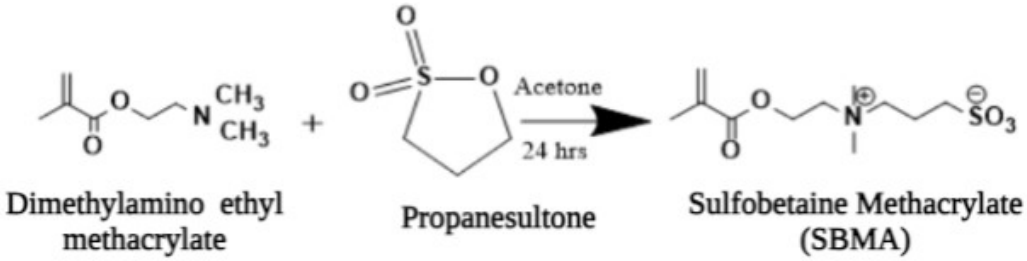
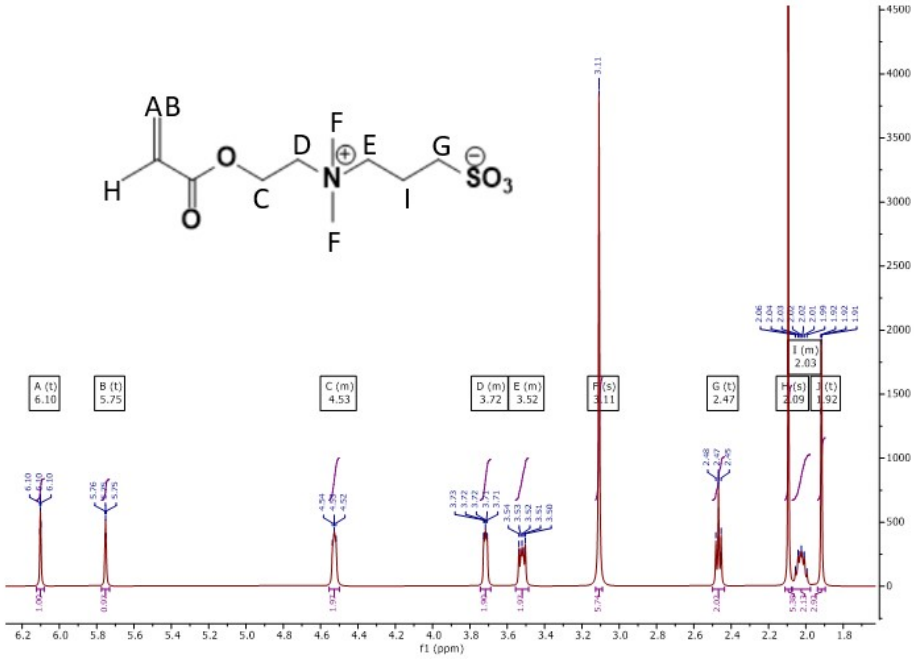
Supplementary Information

Figure S1: Reaction Scheme for SBMA and NMR characterization of SBMA



Scheme1: Synthesis process of SBMA



^{1}H NMR (500 MHz, DMSO-d_6) δ 6.10 (t, J = 1.3 Hz, 1H), 5.75 (t, J = 1.6 Hz, 1H), 4.55 – 4.50 (m, 2H), 3.74 – 3.69 (m, 2H), 3.56 – 3.49 (m, 2H), 3.11 (s, 6H), 2.47 (t, J = 7.1 Hz, 2H), 2.09 (s, 5H), 2.07 – 1.98 (m, 2H), 1.92 (t, J = 1.3 Hz, 3H).

Figure S2: MTT assay of designed bi-layered nanofibrous scaffold for 24 hours and 72 hours

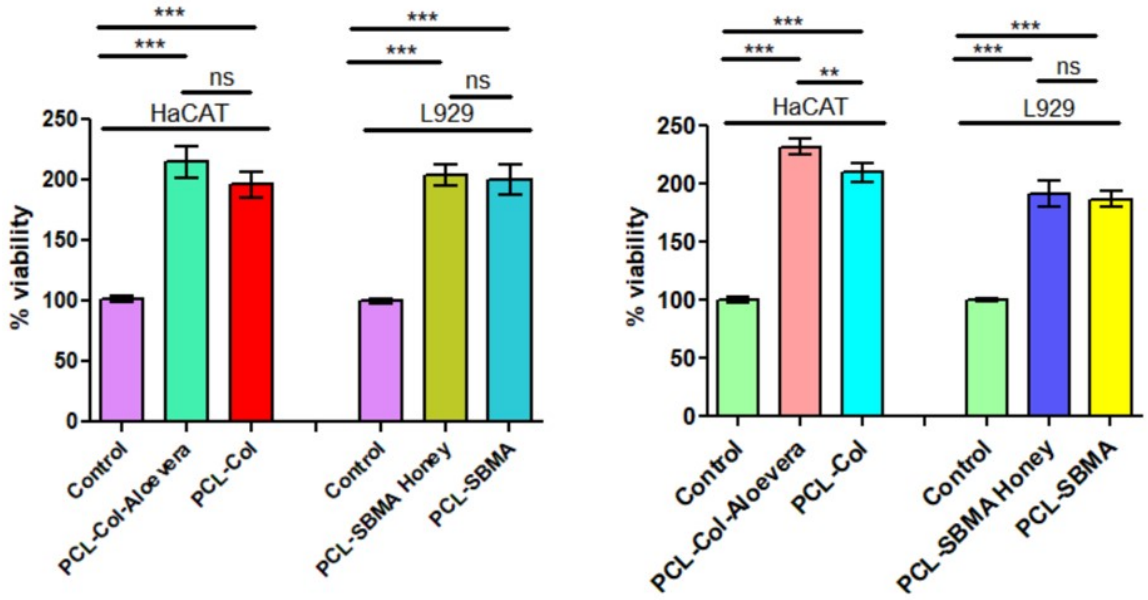


Figure S2: MTT assay of skin cells cultured on the surface of scaffold for 24 hours and 72 hours. ** and *** represents p <0.01 and 0.001 respectively. MTT assay for different time points showed that the herbal extract loaded synthesized scaffold depicted more biocompatibility than the scaffold without herbal extracts, whereas both showed the significant biocompatibility.

Figure S3: DAPI staining of the skin cells cultured on the surface of scaffold for 24 hours and 72 hours

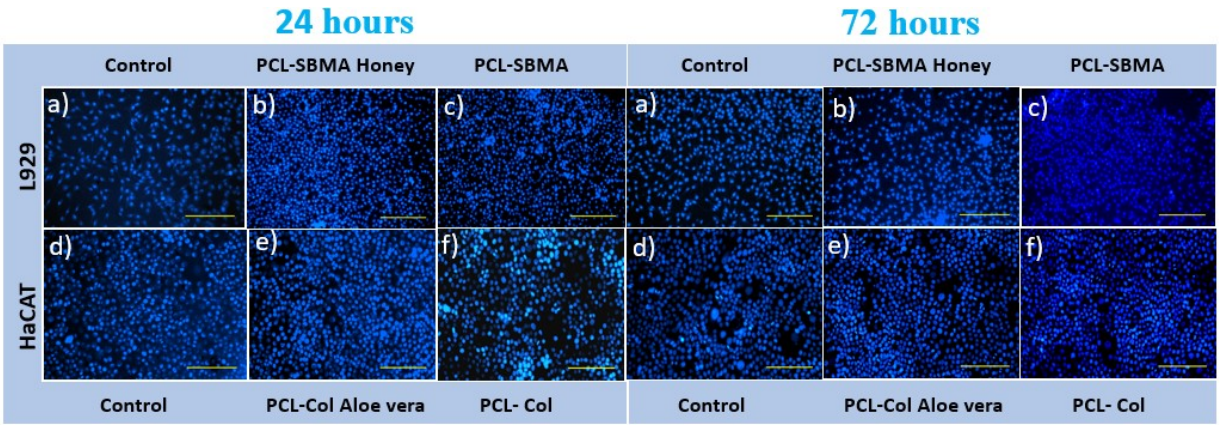
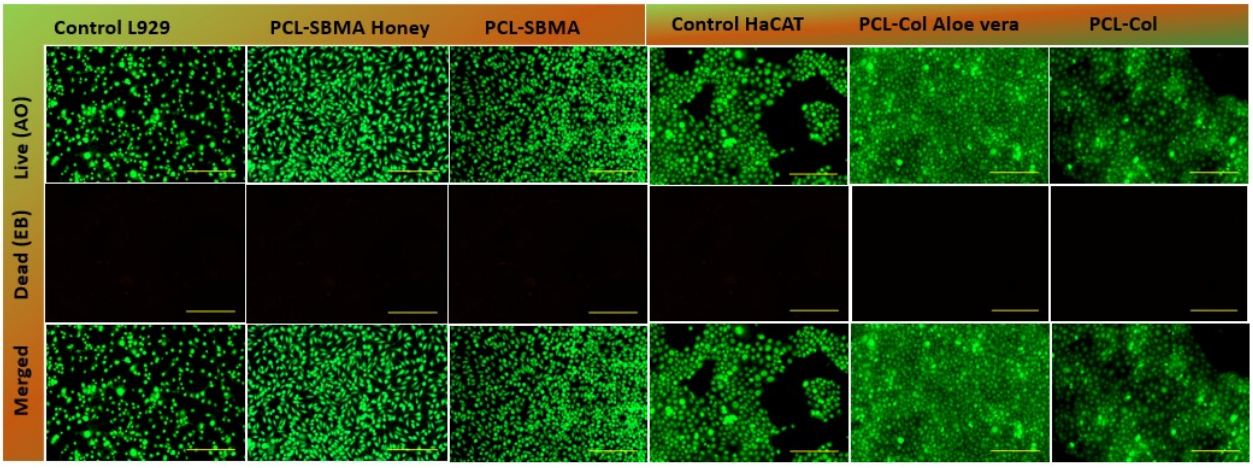


Figure S3: DAPI staining of scaffold for 24 and 72 hours. a) Control L929 b) PCL-SBMA honey c) PCL-SBMA d) Control HACAT e) PCL-Col Aloe vera f) PCL-Col (Scale bar= 200µm). These DAPI stained images demonstrated that the higher population density of both keratinocytes and fibroblast cells were seen on the aloe vera and honey loaded individual layers than the layers without having herbal extracts and also TCP control.

Figure S4: Live-Dead staining of skin cells cultured on surface of the scaffold for 24 hours and 72 hours



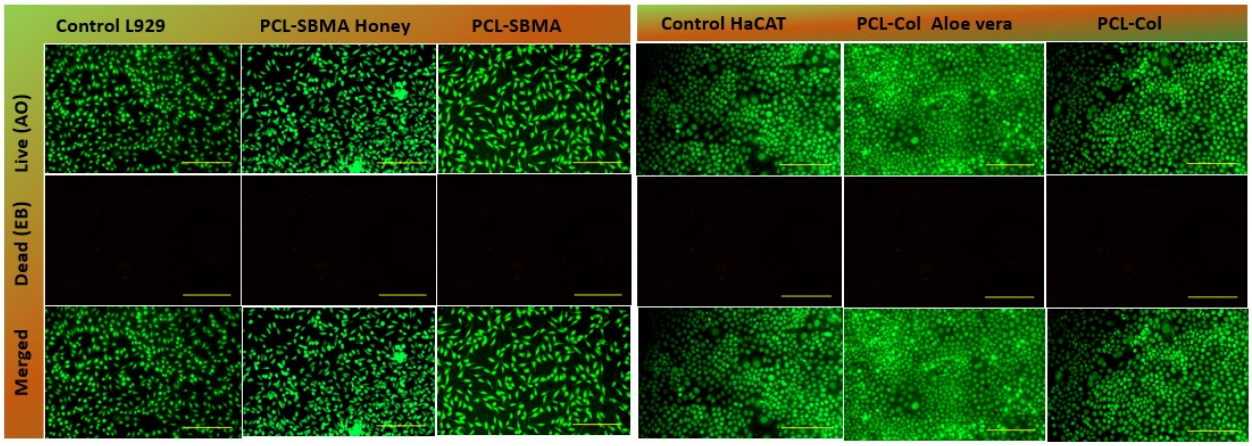
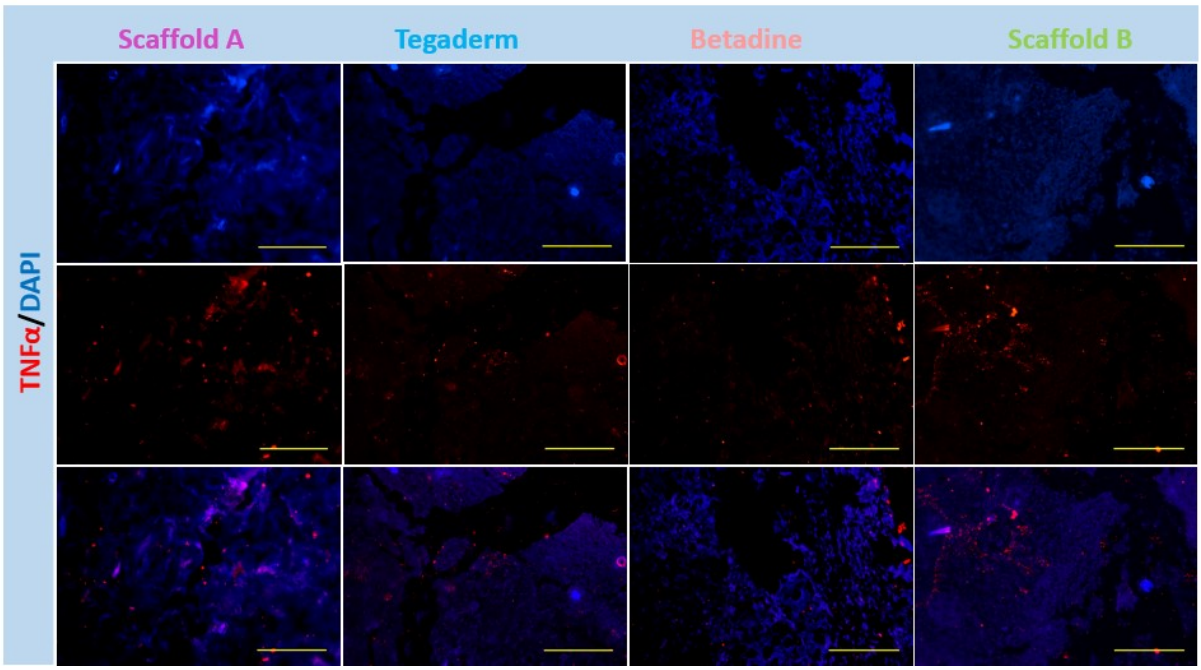


Figure S4: Live-Dead staining images of skin cells cultures on surface of scaffold for 24 hours and 72 hours. (Scale bar=200µm). All of these images for both these time interval demonstrated that both the cells maintained their cellular shape and showed green florescence on the surface of both the scaffolds.

Figure S5: TNFα staining images of different dressings for day 3 and day 7



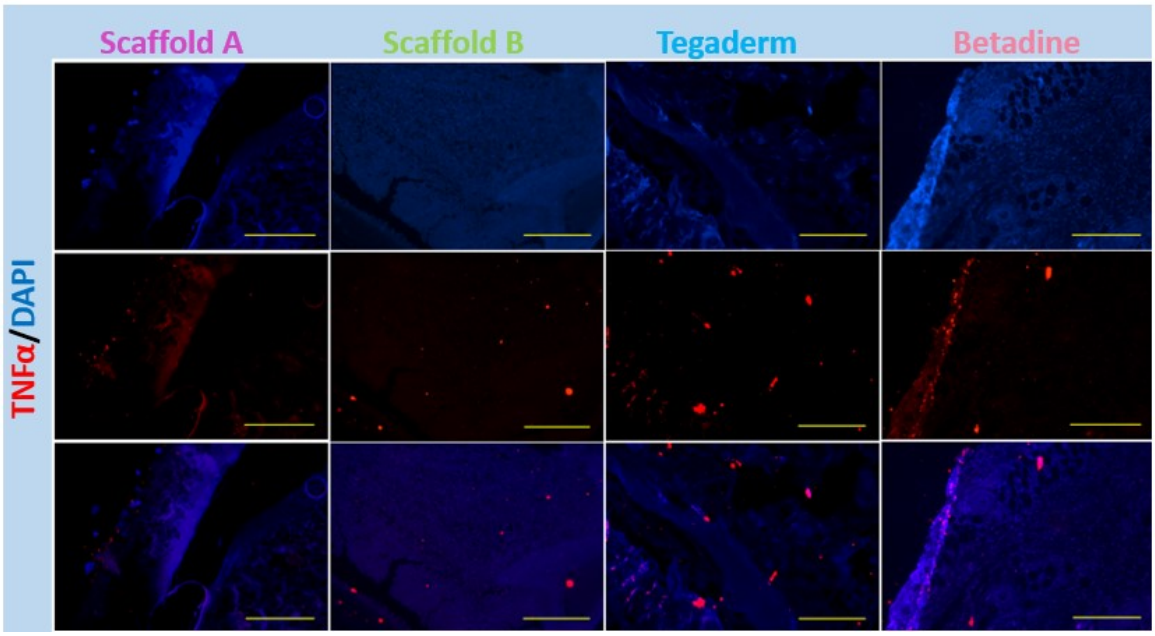
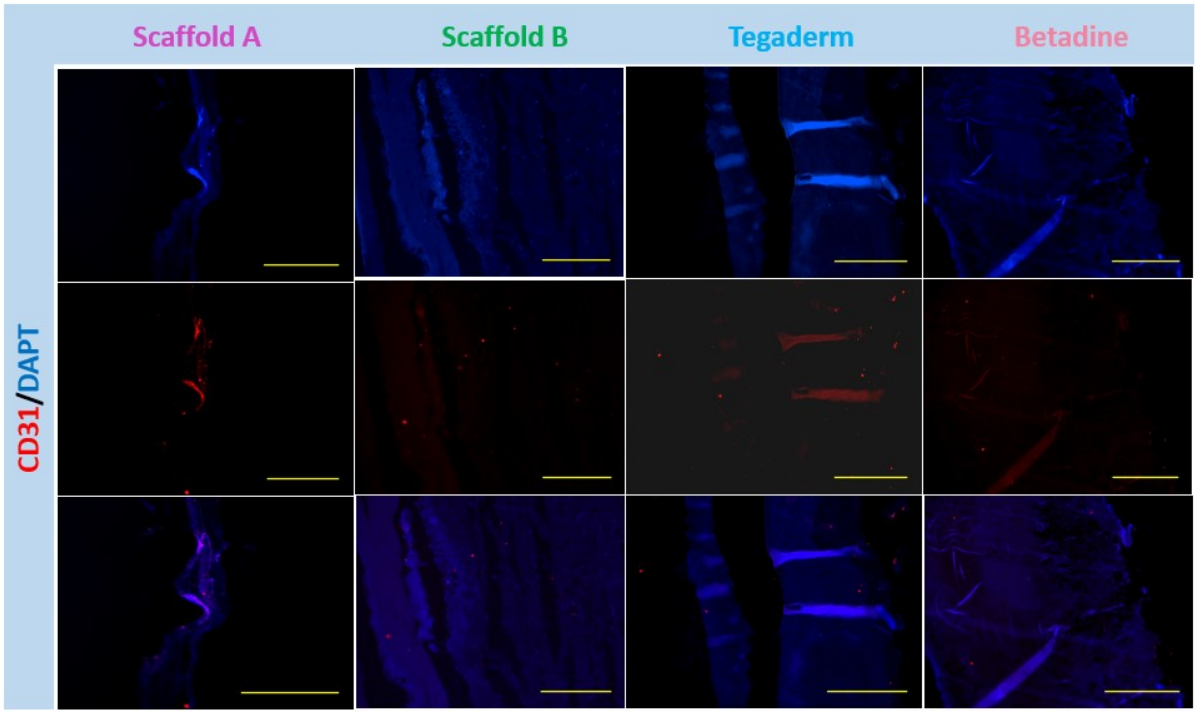


Figure S5: Images of TNF α stained marker of sections for different groups on day 3 and day 7 (Scale bar=200 µm). Inflammatory marker stained fluorescence images of the skin sections showed increased expression in betadine and tegaderm group on day 3 and 7,whereas the expression was found decreasing and a little less in scaffolds group especially herbal extracts loaded group depicting their anti-inflammatory activity.

Figure S6: CD31 stained images for dressings on day 3 and day 7



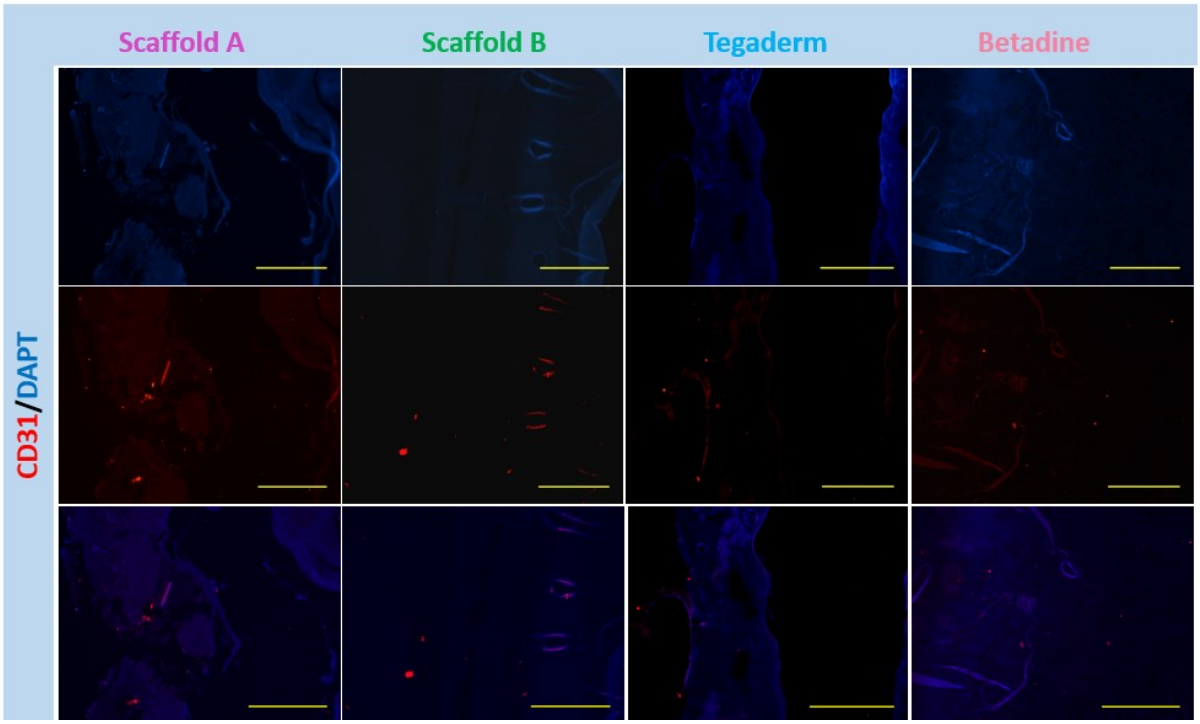


Figure S6: CD31 stained images of sections for different groups on day 3 and day 7 (Scale bar=200 µm). Angiogenesis marker stained fluorescence images for skin sections showed enhanced expression in the case of scaffold A group i.e. aloe vera and honey loaded nanofibrous scaffold compared to other groups.

Figure S7: Histopathological staining of vital organs of rat sacrificed on day 3 and 7.

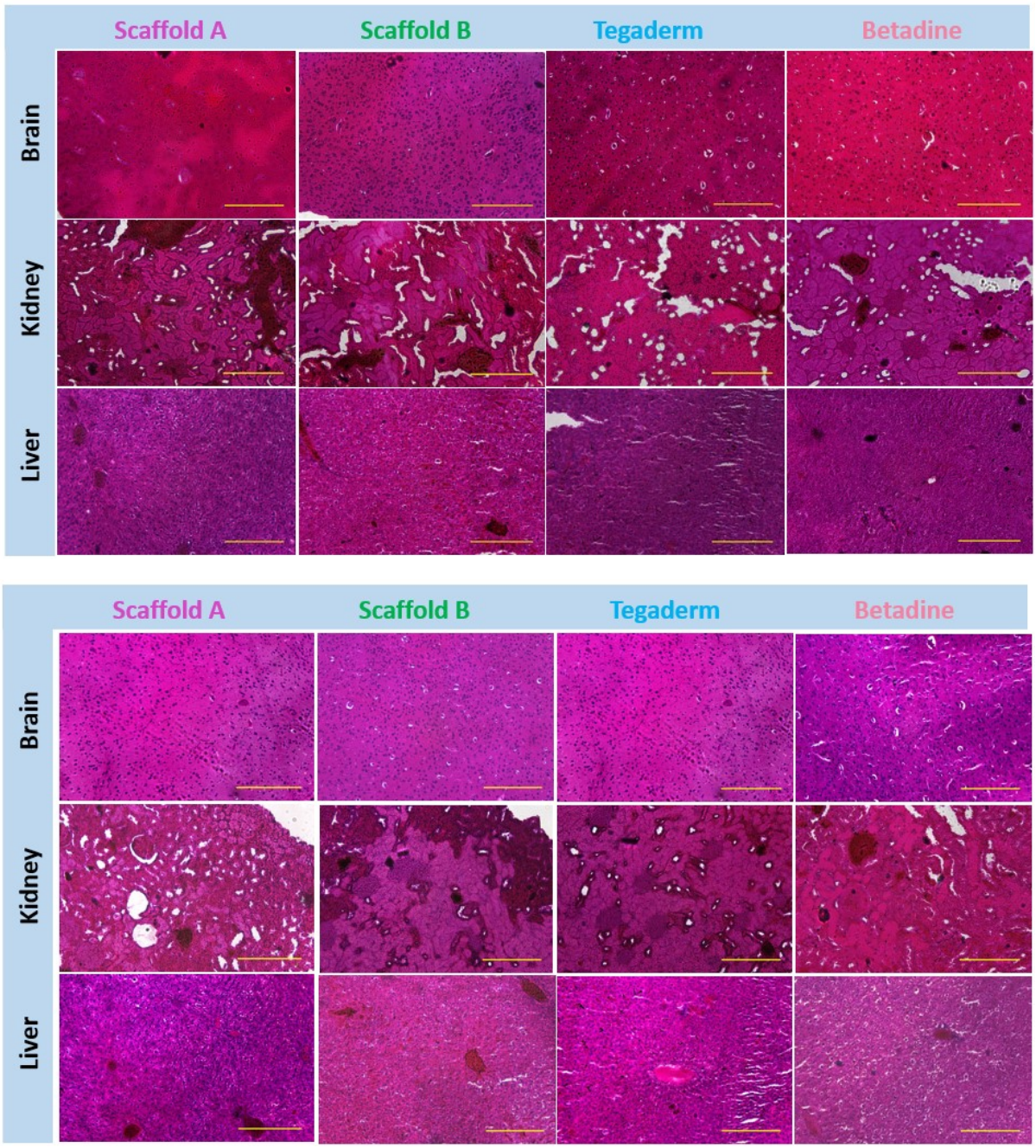


Figure S7: H&E stained images of brain, liver and kidney of rat sacrificed on day 3 and 7 (Scale bar=200 µm). These images showed no adverse effects on the vital organs of rat, confirming the *in vivo* biocompatibility of the designed nanofibrous scaffold.