Supporting Information

Silk Sponges Ornamented with Placenta-Derived Extracellular Matrix Augments Full-thickness Cutaneous Wound Healing by Stimulating Neovascularization and Cellular Migration

Arun Prabhu Rameshbabu[¥], Kamakshi Bankoti[¥], Sayanti Datta[¥], Elavarasan Subramani[$], Anupam Apoorva[*], Paulomi Ghosh[¥], Priti Prasana Maity[¥], Padmavati Manchikanti[α], Koel Chaudhury[$] and Santanu Dhara[¥#]

[¥]Biomaterials and Tissue Engineering Laboratory
School of Medical Science and Technology
Indian Institute of Technology Kharagpur
Kharagpur–721302, India

[$]School of Medical Science and Technology
Indian Institute of Technology Kharagpur
Kharagpur–721302, India

*School of Bio Science
Indian Institute of Technology Kharagpur
Kharagpur–721302, India

[α]School of Energy Science & Engineering
Indian Institute of Technology Kharagpur
Kharagpur–721302, India

#corresponding author
Dr. Santanu Dhara
E-mail: sdhara@smst.iitkgp.ernet.in

S1. Collagen and GAGs Quantification

The total collagen content present in native placenta (NP) and pECM were quantified by hydroxyproline assay kit (Sigma-Aldrich, USA) following the manufacturer's protocol. Briefly, the acid/pepsin soluble collagen in NP and pECM (n = 5) was extracted by digesting with 0.5 M acetic acid containing 1% (w/v) pepsin (Sigma-Aldrich, USA) for 24 h at room temperature. The resulting suspension was centrifuged, and the supernatant was collected for collagen quantification.

Sulfated GAGs (sGAGs) present in NP and pECM was extracted by digesting with 10 mM cysteine hydrochloride (Sigma-Aldrich, USA), 125 mg/mL papain (Sigma-Aldrich, USA) and 2 mM EDTA (Sigma-Aldrich, USA) at 60 °C for 60 h. The sGAGs content of NP and pECM (n=5) was assessed by alcian blue assay as described elsewhere.[25] Further, sGAGs present in samples was quantified via the standard curve obtained with different concentrations of chondroitin sulfate A (Sigma-Aldrich, USA).

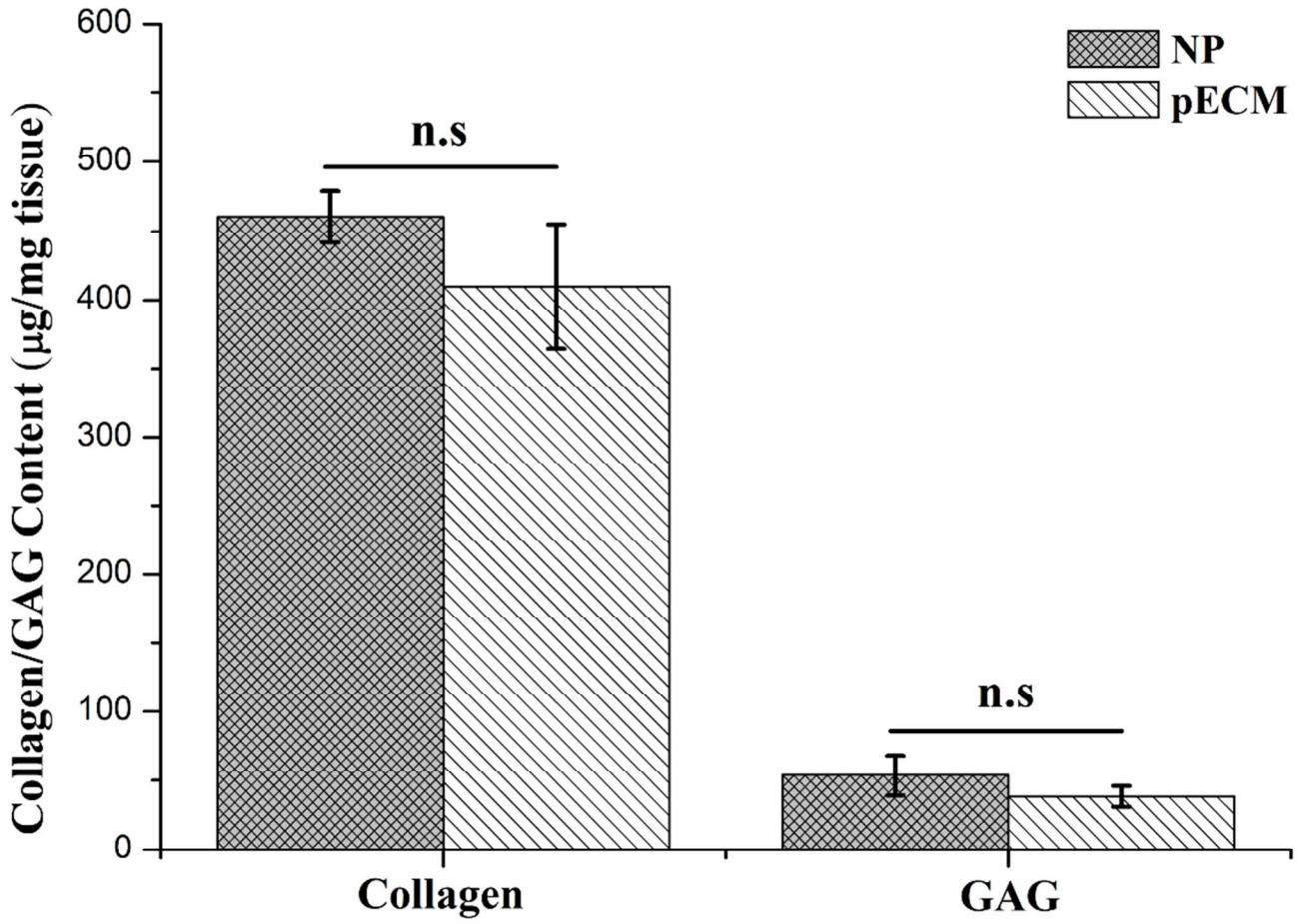

Figure S1: Collagen/GAG quantification of NP and pECM

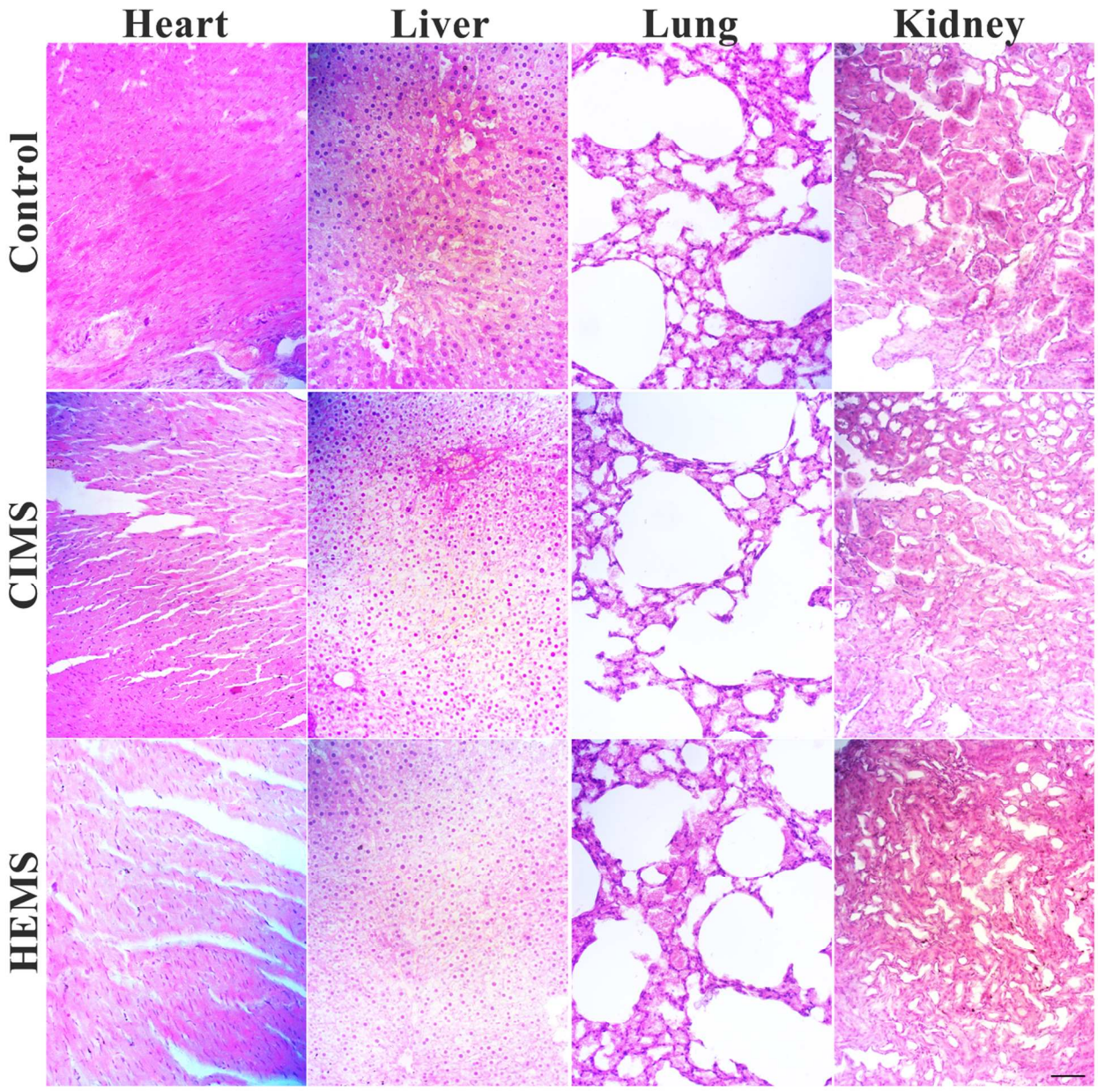

Figure S2: H & E staining of heart, liver, lung and kidney harvested from control, CIMS and HEMS treated group after three weeks post-treatment (scale bar represents 50 µm).

S3. Masson's Trichrome Staining of Hybrid Scaffolds

The fabricated silk fibroin (SF)/CIMS/HEMS were fixed in 4 % paraformaldehyde for 30 minutes, followed by dehydration. The dehydrated samples were embedded in paraffin blocks and sectioned using microtome. The sections were stained with Masson's trichrome (MT; Sigma-Aldrich, USA) according to the manufacturer's instructions.

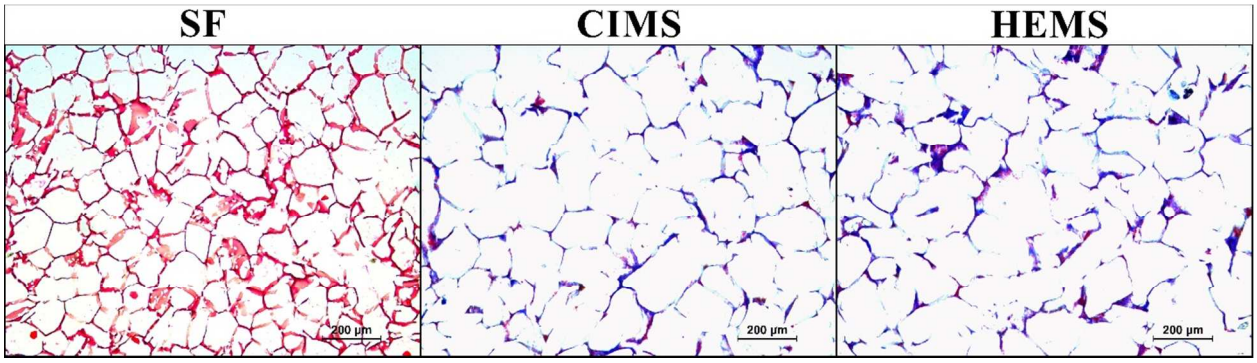

Figure S3: MT staining of the SF and hybrid scaffold (CIMS/HEMS) demonstrating uniform distribution of collagen/pECM (blue color in CIMS/HEMS signifies the presence of collagen; whereas it is absent in pristine SF scaffolds).

Table S1: Gene Specific Primers for Reverse Transcriptase-PCR (RT-PCR)

Name of Gene	Sequence	Fragment Size (Bp)	Tm (°C)
GAPDH	F-CCATGGAGAAGGCTGGGG R-CAAAGTTGTCATGGATGACC	195	54
COL I	F-GCGCCAGAAGAACTGGTACATCAGCAA R-AAGCGTTTGCGTAGTAATTGCA	100	60
COL III	F-CTGAAATICTGCCATCCTGAAC R-GGATIGCCGTAGCTAAACTGAA	236	58.4
KRT 10	F-CATGAGTGTCCCCCGGTATC R-CAGTATCAGCCGCTTTCAGA	79	59
KRT 14	R-GCGCGCCATACTCGAACTGGAATC F-TTCTCACAGCCACAGTGGAC	281	59