Supporting Information

Carbon Nanodot Decorated Acellular Dermal Matrix Hydrogel Augments Chronic Wound Closure

Kamakshi Bankoti[a], Arun Prabhu Rameshbabu[a], Sayanti Datta[a], Madhurima Roy[b], Piyali Goswami[b], Pallab Datta[c], Amit Kumar Das[b], Sudip Kumar Ghosh[b], Santanu Dhara[a]

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

[b]Department of Biotechnology
Indian Institute of Technology Kharagpur
Kharagpur – 721302, India

[c]Centre for Healthcare Science and Technology,
Indian Institute of Engineering Science and Technology
Shibpur, Howrah 711103, India

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

S.1. Quantification of GAG and Collagen. GAGS were extracted and quantified from NS and ADM ($n = 5$) using modified AB assay.[1] The GAGs were isolated by digesting NS and ADM with papain enzyme (125 μg ml^{-1}) in phosphate buffer solution (0.1 M, pH 6.8) containing cysteine hydrochloride (10 mM) and Ethylene diamine tetra acetic Acid (EDTA, 2 mM) (all chemicals from Sigma-Aldrich, USA) at 60 °C for 60 h. Subsequently, undigested tissue was removed by collected by centrifugation (13, 000 rpm for 20 min) and sample/standard solution were mixed with working solution of AB. The absorbance was measured using an iMark™ microplate reader at a wavelength of 595 nm. GAGs in samples was estimated from the standard curve using chondroitin sulfate A (Sigma-Aldrich, USA).

Collagen was measured using the hydroxyproline assay described elsewhere.[2] In brief, lyophilized tissue (NDM and ADM) was hydrolyzed using 6M HCl (10mg tissue/ml) in an autoclave (120 ^{0}C for 2-4 h), followed by drying and neutralization. Standards are prepared, and to both sample & standard, Chloramine-T was added, which was allowed to react for 30 min. Subsequently after addition of 1 ml of aldehyde-perchloric acid reagent, heated at 60 ^{0}C for 15min. Samples (n=3) were brought to room temperature, and absorbance was recorded at 550 nm. Sample concentration was determined from the standard curve.

S.2. DPPH Assay. Free radical scavenging property of the hybrid hydrogel was evaluated using *ex vivo* DPPH (Sigma Aldrich, USA) assay as described in previous reports.[3] Briefly, 0.2 mg ml^{-1} of methanolic DPPH (300 μl) solution was added to the samples/ standard (butylated hydroxyl toluene (BHT)) and incubated in the dark for 1 h 40 min. After 1 h 40 min., OD was measured by using a UV-Vis Spectrometer (Shimadzu, Japan) at 517 nm. Scavenging (AA) % was calculated by

$$AA\% = 100 - \frac{(Absorance\ Sample - Absorance\ Blank)}{Absorbance\ Control} X100$$

S.3. Hemolytic assay. Hemocompatibility of hydrogels was evaluated using direct contact hemolysis assay.[4] Briefly, uncoagulated blood (3% sodium citrate) was added to sterile saline (1:1). A different variant of the hydrogel of similar weight was incubated with 1 ml uncoagulated blood at 37 °C for 60 min, and the supernatant was collected by centrifugation. OD of the supernatant was measured using Multiskan Spectrophotometer (MK3, Thermo Scientific, USA) at 540 nm. Normal saline was used as positive control and 1 % Triton-X™ was used as negative control for the study.

% Haemolysis= OD of RBC treated Hydrogel-OD of RBC treated normal saline)/ (OD of RBC treated triton X™ - OD of RBC treated normal saline) X 100

S.4. Stem Cells and fibroblast isolation. HAMSCs/Fibroblast were isolated according to our previous work[5] after taking approval of the Ethical Committee of Indian Institute of Technology, Kharagpur, India. Briefly, fresh human placentas were collected under the stringent sterile condition and were washed several times with Hank's Balanced Salt Solution (HBSS medium, Gibco, USA) containing antibiotic and antimycotic (Invitrogen, ThermoScientific, USA). Amniotic membrane was dissected from chorion and digested using 0.05 % trypsin–EDTA solution (Gibco, USA) for removal of amniotic epithelial cells for two cycles 30 min each. The supernatant obtained was discarded, and tissue was washed with Earle's Balanced Salt Solution (EBSS, Gibco, USA) to remove remaining Trypsin in tissue. Tissue obtained was further digested using DNase I (10 U ml^{-1}, Sigma-Aldrich, USA) and collagenase Type IV (2 mg ml^{-1}, Gibco, USA) for 60 min. Post digestion cell pellet was collected using centrifugation and suspended in complete low glucose media (Gibco, USA) for seeding in a flask, followed by transferring in an incubator (37 °C, 5% CO_2). Post 80 % confluency the flasks were passaged using 0.25% trypsin–EDTA (Gibco, USA) and cells after passage 2 was used for the study.

For fibroblast isolation human foreskin tissue was collected freshly from volunteer post written consent and washed several times with PBS containing penicillin (200 U ml^{-1} Gibco, USA) and streptomycin (200 μg ml^{-1} Gibco, USA) to get rid of adhering blood and tissue. The tissue was cut into small pieces and incubated in Dispase II solution (Sigma-Aldrich, USA) at 4 °C for 14-16 h. After incubation, the epidermis was removed from the dermis and discarded. Dermis so obtained was washed several times with sterile PBS and digested using collagenase I solution (Gibco, USA) at 37 °C for 2-3 h. The supernatant was collected by filtering through tissue strainer (100 μm) and the supernatant was centrifuged to collect cell pellet. The cell pellet was suspended in complete high glucose medium (Gibco, Invitrogen, USA) and seeded in the flask. Passaging was done after 80 % confluency was obtained using 0.25 % trypsin and cells were used after the second passage for experiments.

S. 5. Gene Expression. Animals were sacrificed by an overdose of anesthesia post 21 days of wounding, and healed tissue (n=3) were retrieved. RT-PCR was performed on retrieved tissue of all groups. In brief, tissue retrieved was snap froze by dipping in liquid nitrogen and crushed to powder. Total RNA was isolated using TRIzol reagent (Invitrogen, USA), followed by transcribing to cDNA using a cDNA synthesis kit (Thermo Fisher Scientific, USA) using the protocol provided by the manufacturer. PCR gene amplification of specific primers was performed using cDNA in a thermal cycler (Eppendorf Mastercycler, USA). PCR product was

resolved using agarose gel (1%), the picture was taken using UV Gel doc (Bio-Rad, USA), and subsequently quantification with Image J (Rasband WS; NIH).

Table S.1 *Primer Sequences*

Gene	Forward primer	Reverse primer
Keratin 10	5'-CAGATAGGCCAGCTCTTCAGT-3'	5'-GACATCAACGGCCTGCGTA-3'
Collagen I	5'-ACATGTTCAGCTTTGTGGACC-3'	5'-CATGGTACCTGAGGGCGTTC-3'
Collagen III	5'-ATGTTGTGCAGTTTGCCCAC-3'	5'-TCGTCCGGGTCTACCTGATT-3'

RESULT:

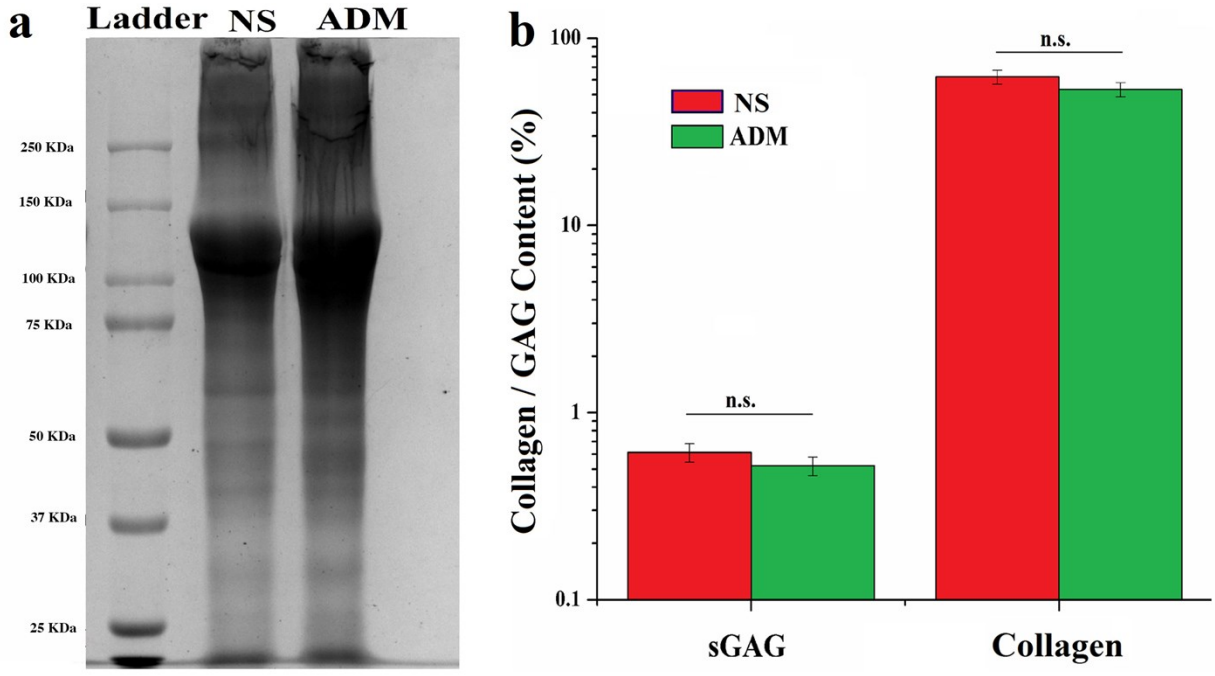

Fig. S1 (a) Total protein extracted from NS and ADM was separated by SDS-PAGE and (b) biochemical analysis of Col and glycoaminogycans (GAG) in NS and ADM. Y-error bars represent standard deviation.

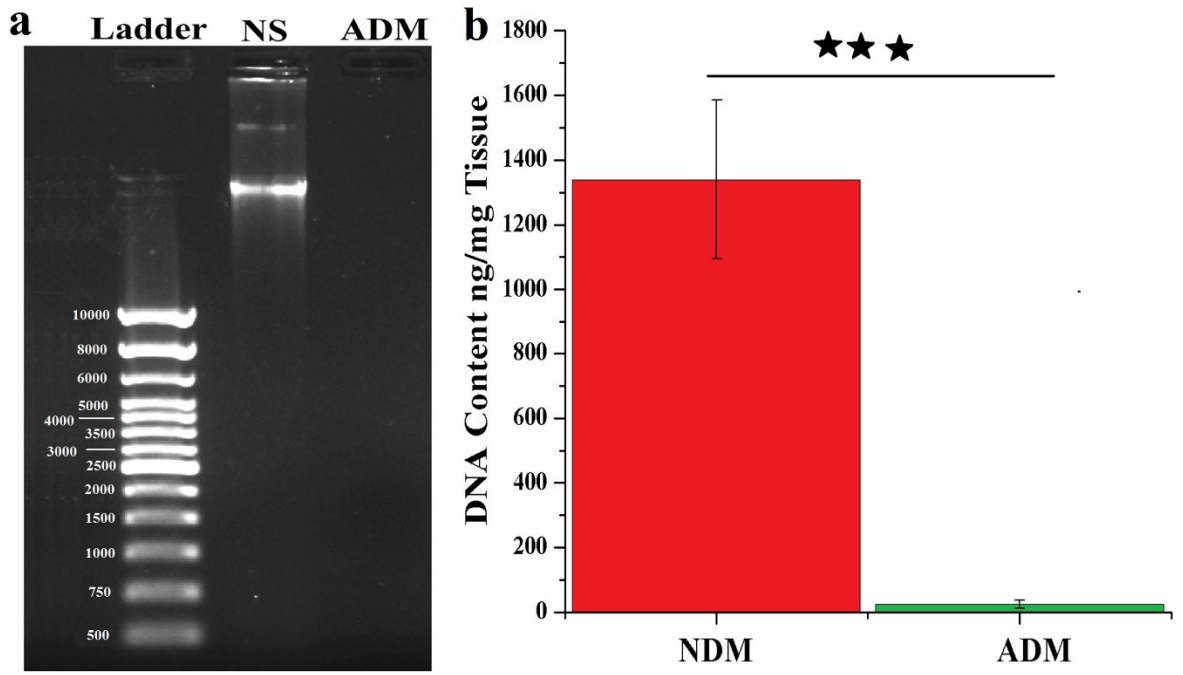

Fig. S2 (a) Agarose gel electrophoresis of the isolated DNA from NS and ADM in 1% gel and, (b) DNA quantification. Y-error bars represent standard deviation; *** represents $p<0.001$.

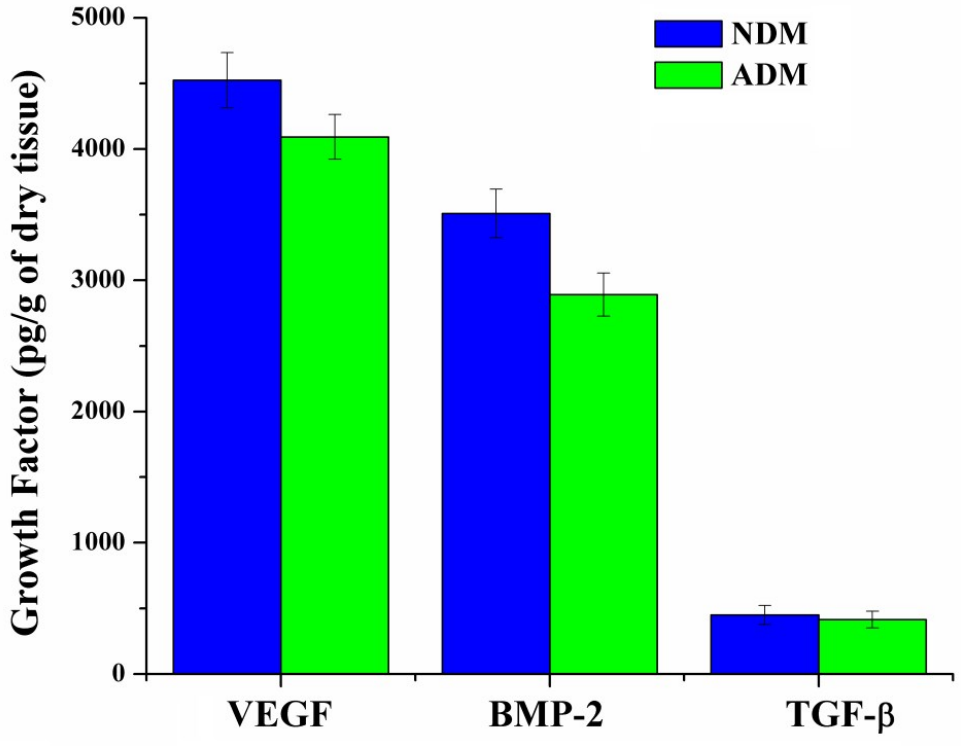

Figure S3 Quantitative analysis of different growth factors (VEGF, TGF-β and BMP-2) present in NS and ADM using ELISA. Y-error bars represent standard deviation.

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