Supporting Information

The Smart Piezoelectric Nanohybrid of Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) and Barium Titanate for Stimulated Cartilage Regeneration

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1. Optimization of electrospinning parameters

The electrospun scaffolds of pure and composite were prepared by electrospinning method using E-SPIN NANO (PECO, Chennai , India) electrospinning system. The optimization of parameters such as the concentration of the solution, flow rate, applied voltage and working distance (distance between needle tip and collector) is essential to tune the fibre diameter. Generally, the optimization is done by varying of one parameter (voltage) at others kept constant i.e., concentration: 15 %, flow rate: 1 mL/hr, working distance: 15 cm at different voltages **(**12.5, 15, 17, 20, 25, 30 and 35 kV). Similarly, all other parameters such as concentration, flow rate and working distance were optimised at other factors at constant. The optimisation of nanofibers was accomplished by observing the fibre diameter through optical microscopy (MOTIC BA 210) as shown in fig.S1. Table S1 shows the optimisation parameters of the pristine polymer and its various composites.

Table S1. The optimized parameters for electrospinning of pristine polymer and its BT nanohybrids

Concentration	Flow rate (mL/hr)	Working distance (cm)	Voltage (kV)
PB	1	20	20
PB15-BT5	1	20	20
PB15-BT10	1	20	20
PB15-BT20	1	20	20
PB20-BT5	1	20	20
PB20-BT10	1	20	20
PB20-BT20	1	20	20

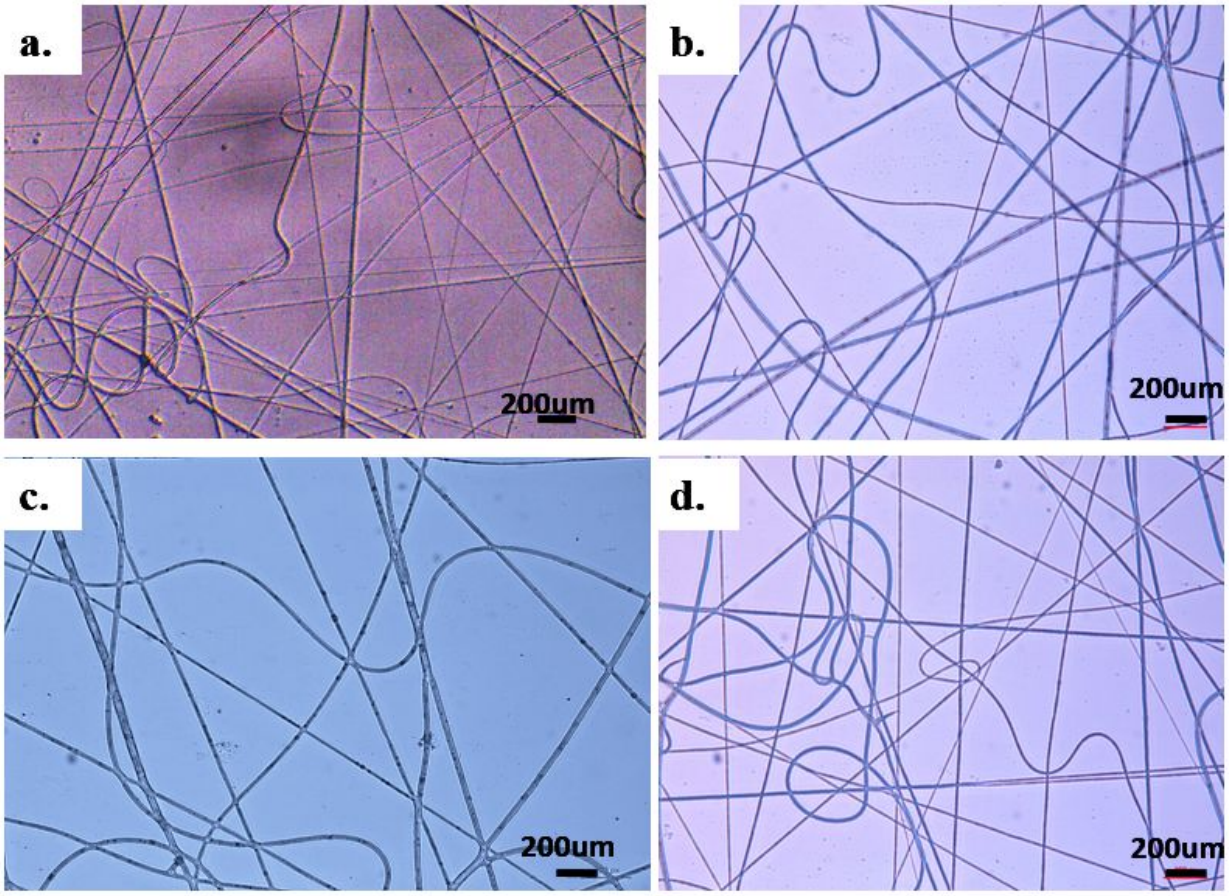

Fig.S1. Optical microscopy images of the samples: (a) PHBV (b), (c) & (d) 15% and 20% PB with different concentrations of BT.

2. Calculation of voltage from piezoelectric coefficient (d_{33})

The observed voltage in PB20-BT-20 was ~ 50mV/mm and it was calculated from the following equations

$$g_{33} = \frac{d_{33}}{\epsilon_o k} \text{ and } V = \frac{g_{33\,FL}}{A}$$

Here d_{33} is piezoelectric coefficient, g_{33} is voltage coefficient, ε_o is permittivity of free space, k is the relative dielectric coefficient V is the voltage, F is the applied force L is the length of the specimen and A is the cross section area of the specimen

3. Differentiation of mesenchymal stem cells (MSCs)

The differentiation of the MSCs into chondrocytes was carried out as per the HiMedia protocol [1] Initially, MSCs were incubated for 28 days in chondrogenic differentiation medium (HiChondroXL^{TM}) as suggested by the protocol. The differentiated cells were confirmed by safranin staining, (specific for proteoglycan), sirius red staining, (specific for collagen II) and by RT-PCR using RNA isolated from the differentiated cells. It is well documented that the chondrocytes are most unstable and lost chondrocytic features rapidly by dedifferentiation to proliferation [2]. Further, they undergo multiple divisions and passaging during the *in vitro* proliferation process and inferior potential to replicate themselves [3]. These restrictions prompted to select another source for tissue engineering. The clonally expanded MSCs have pluripotential capacity to differentiate into a variety of connective tissue lineages including adipocytes, osteocyte and chondrocyte [4]. The MSCs are cultured in polypropylene centrifuge tubes for differnition and maintained predefined conditions. Fig.S2 shows the aggregate form of cells after 1 and 15 days of incubation (tube) and the microscopic image clearly shows the chondrocytes after 28 days incubation.

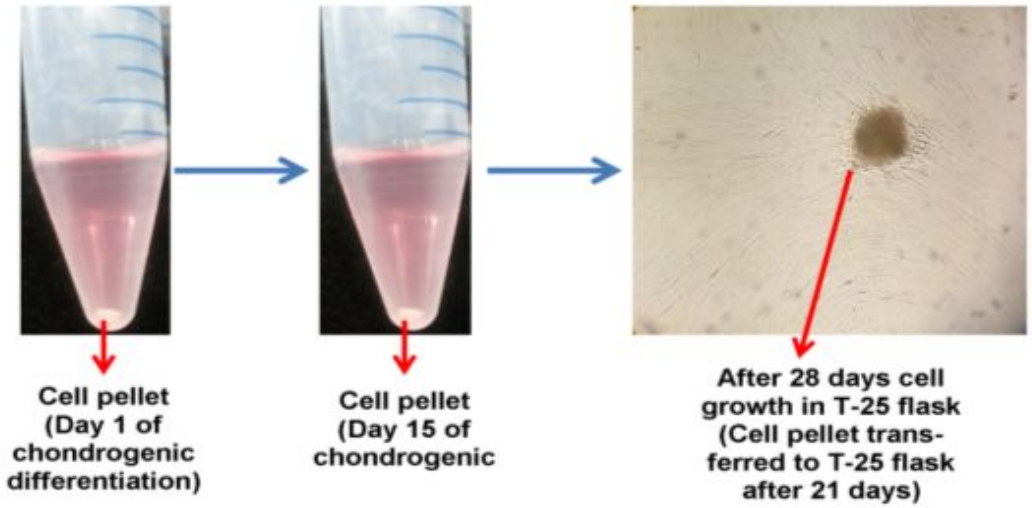

Fig.S2. The optical images of chondrogenic differentiation in a centrifuge tube and cell growth in T-25 flask at 28th day of post-incubation.

Confirmation of the Chondrocyte

3.1. Safranin staining:

The proteoglycan is the matrix marker of chondrocytes. Safranin can strongly stain the proteoglycan. The staining was done with both MSCs and differentiated chondrocytes and the imaging was done by AXIO Observer A1 Model microscope (ZEISS). The fig.S3a depicts strong positive stain in chondrocyte culture than the MSCs culture (fig.S3b), confirming the presence of proteoglycan marker in the chondrocyte cell culture.

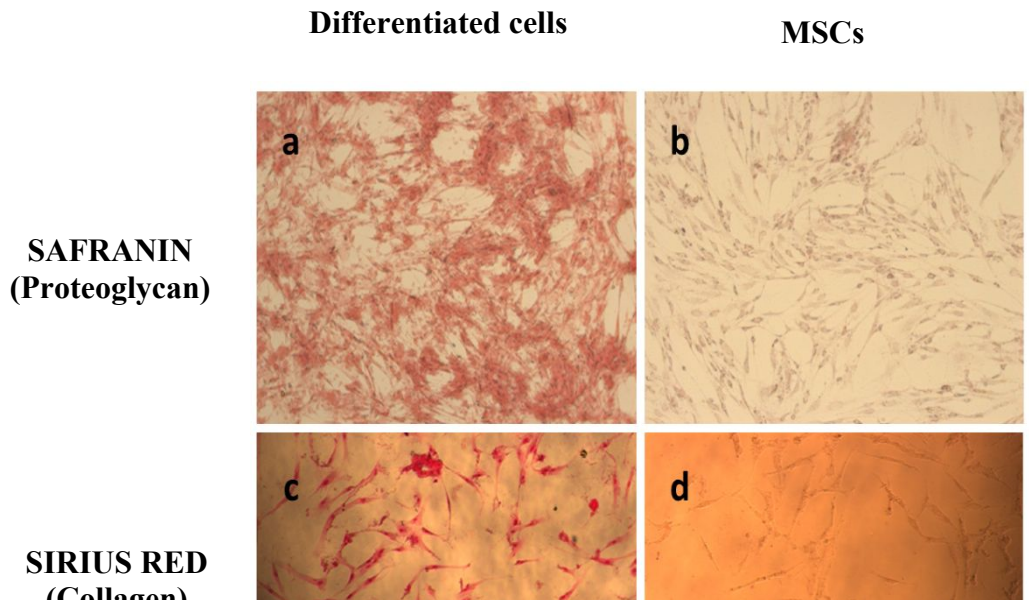

Fig.S3. Matrix synthesis detected after 28 days of chondrogenic induction. a) & b) representing the safranin staining on differentiated cells and MSCs, respectively. Images c) & d) represents the sirius red staining on differentiated cells and MSCs respectively. (scale bar = 100 μm)

3.2. Sirius red staining:

Type II collagen is the principal constituent in collagen found in physiological hyaline cartilage and it provides tensile strength to pass the functional stresses without friction at joint [5]. Sirius red dye is a robust anionic dye which stains collagen by reacting via its sulphonic acid groups present in the collagen molecule. The staining was done on both MSCs and differentiated cells. According to the results (Fig.S3.c & d), the differentiated cells are showing the more considerable staining than MSCs. Both Safranin and Sirius red shows the positive stain for differentiated cells than MSCs. The proteoglycan and collagen are two essential components of the ECM of cartilage and are synthesized by chondrocytes. Thus, the differentiation of MSCs to chondrocytes can be confirmed from the above results.

3.3. Confirmation by RT-PCR

Further, chondrocytes have been confirmed by their selective markers such as SOX9 and COL2A1. It is evident that chondrocytes have shown strong expression for both the markers (Fig.S4), while

absent in MSCs. Thus, the differentiation of MSCs to chondrocytes have been confirmed qualitatively.

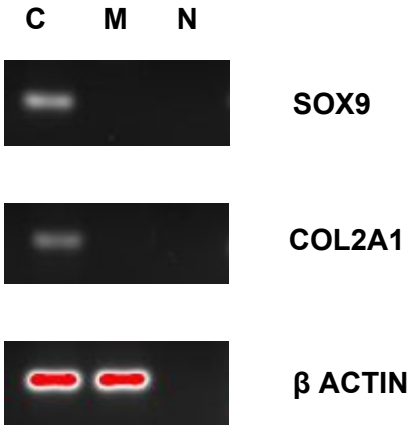

Fig.S4. Expression of SOX9, COL2A1, and β-actin in differentiated cells (chondrocytes) and MSCs as evaluated by agarose gel electrophoresis of amplified products. C: Chondrocytes, M: mesenchymal stem cells, N: Non- template control.

References

[1] http://himedialabs.com/TD/AL523.pdf.
[2] Chen WH, Lai MT, Wu AT, Wu CC, Gelovani JG, Lin CT, et al. In vitro stage-specific chondrogenesis of mesenchymal stem cells committed to chondrocytes. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2009;60:450-9.
[3] Im GI, Jung NH, Tae SK. Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. Tissue engineering. 2006;12:527-36.
[4] Cheng NC, Estes BT, Awad HA, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells by a porous scaffold derived from native articular cartilage extracellular matrix. Tissue Engineering Part A. 2008;15:231-41.

[5] Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. Clinical orthopaedics and related research. 2001;391:S26-S33.