

Electronic Supplementary Material

Development and evaluation of a 3D composite scaffolds using piezoelectricity and biofactor synergy for enhanced articular cartilage regeneration

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Gene	Forward (5' -3')	Reverse (5' -3')
GADPH	CAAGAAGGTGGTGAAGCAGG	CACTGTTGAAGTCGCAGGAG
Aggrecan	GGAGGAGCAGGAGTTTGTC	TGTCCATCCGACCAGCGAAA
SOX9	GCGGAGGAAGTCGGTGAAGAA T	AAGATGGCGTTGGGCGAGAT
COL-2	CACGCTCAAGTCCCTCAACA	TCTATCCAGTAGTCACCGCTCT

Table S1 Corresponding RT-qPCR Primers.

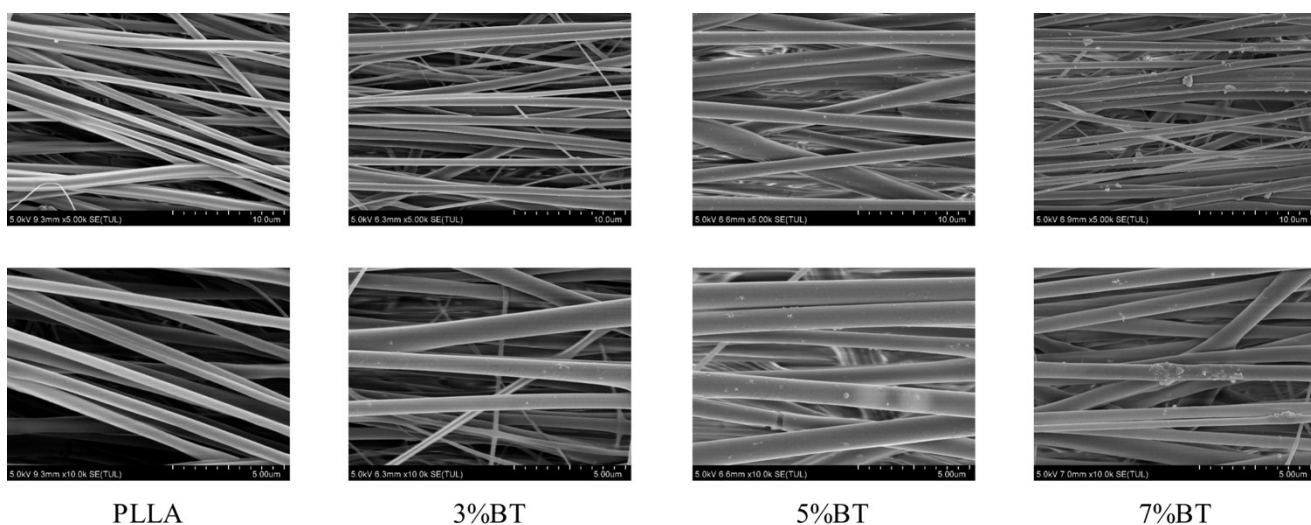


Figure S1 SEM images of PLLA/BT in different ratios.

SEM analysis was performed on pure PLLA, 3% BT, 5% BT and 7% BT samples. **Fig. S1** shows that the neat PLLA nanofibers did not form beads, and the fibers were continuous, smooth, and uniform. Doping PLLA fibers with 3% BT NPs, a small amount of unevenly dispersed BT NPs on the surface can be observed, which directly affects the piezoelectric properties of the nanofiber membrane due to the uneven distribution of BT. When the content of BT NPs was increased to

5%, the PLLA/BT nanofibers were continuous and homogeneous, and no aggregation of BT NPs was formed, showing that the particles were uniformly dispersed in the fiber matrix and the crystal particles were arranged. When the concentration of BT NPs particles was added to 7%, beads were formed on the surface of the nanofiber membrane, and the BT NPs particles appeared to be agglomerated.

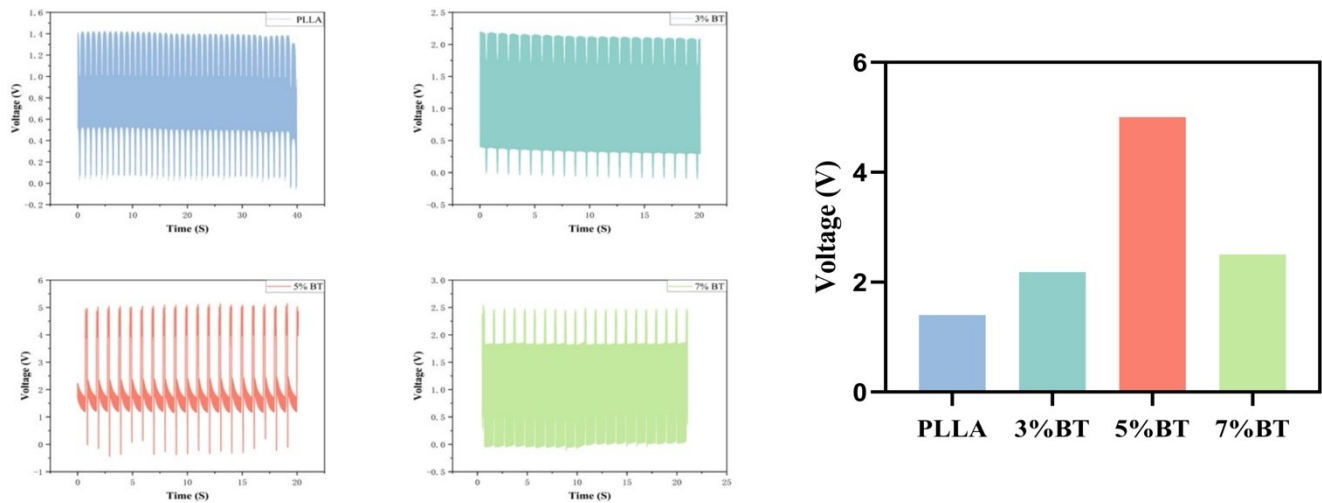


Figure S2 Piezoelectric output for different ratios of PLLA/BT.

The piezoelectric properties of PLLA/BT nanofiber membranes with different ratios are illustrated in **Fig. S2**. It is evident that the piezoelectric output of pure PLLA nanofiber membranes measures about 1.4 V. Upon the addition of BT NPs particles, the piezoelectric output increases: reaching approximately 2.2 V for the 3% BT group and approximately 5 V for the 5% BT group. This increase in piezoelectricity, from 1.4 V to 5 V, corresponds to the BT content ranging from 0 to 5%. Initially, it's essential to note that BT, serving as a representative material of piezoelectric ceramics, boasts a high piezoelectric coefficient of up to 191 pC/N. The BT content significantly influences the piezoelectric output of PLLA/BT composite fiber membranes. However, upon increasing the BT content to 7%, particle agglomeration issues arise. Consequently, the piezoelectric performance of the composite fiber membrane decreases to 2.5 V. This decline indicates a reduction in the interfacial area between BT and PLLA due to agglomeration, thereby affecting the piezoelectric performance of the nanofiber membranes to a certain extent.

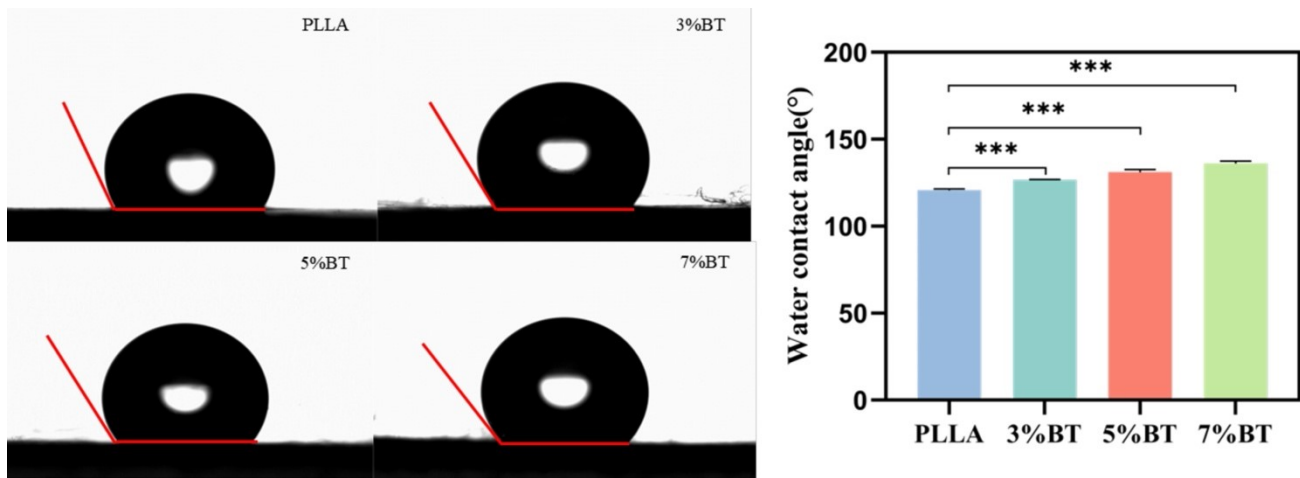


Figure S3 Water contact angle of PLLA/BT in different ratios.

From **Fig. S1c**, it is evident that the water contact angle of the material increases with the rise in BT concentration, indicating a decrease in hydrophilicity. Hence, BT significantly influences the hydrophilicity of the composite material. SEM analysis and piezoelectric output measurements revealed that the composite with 5% BT exhibited continuous and uniform fibers without BT NPs aggregation and demonstrated higher piezoelectric output compared to other concentrations. Consequently, 5% BT was selected for subsequent experiments. Although slightly less hydrophilic than 3% BT, subsequent treatment could enhance the material's hydrophilicity, aligning with our research focus on materials with high piezoelectric output. The level of hydrophilicity did not directly impact our experimental choice.

In summary, 5% BT was chosen as the research subject for subsequent experiments, based on its favorable characteristics observed through SEM analysis and piezoelectric output measurements.

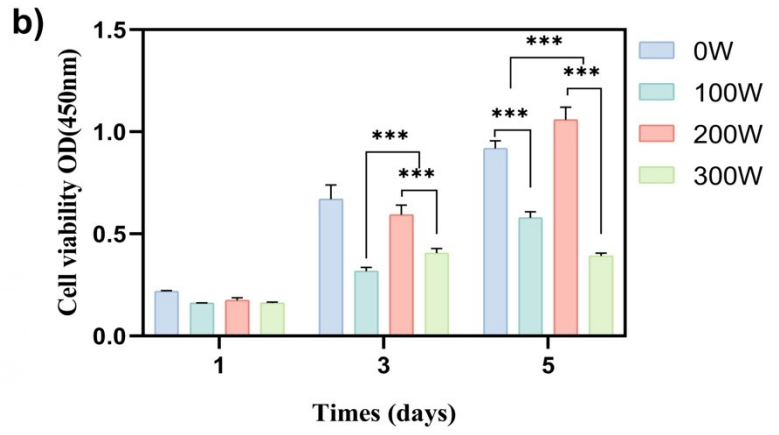
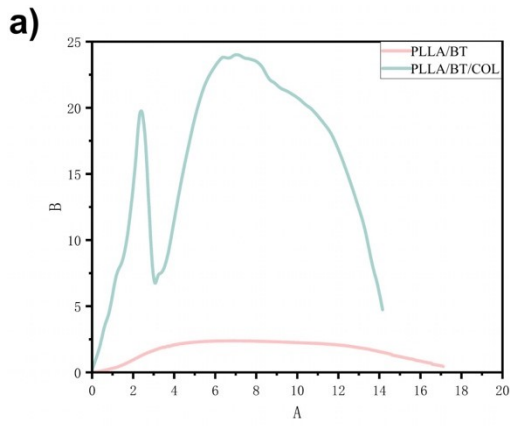


Figure S4 a) Comparison of mechanical properties between PLLA/COL/BT and PLLA. b) Trends in cell proliferation at different ultrasound powers.



Video S1 Video of rabbits exercising on a treadmill 2 weeks after recovering from surgery.

