Poly（L-Lactide –co-Caprolactone）scaffolds enhanced with poly (β-hydroxybutyrate-co-β-hydroxyvalerate) microspheres for cartilage regeneration

Chao Li, Jingjing Zhang, Yijiang Li, Shamus Moran, Gilson Khang, Zigang Ge, *

Supplementary Data

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Materials and Methods:

Water Contact Angle：

Zero point of two gram of PLCL and PHBV polymer were dissolved in 2ml methylene chloride solution. After the methylene chloride was evaporated completely, the PLCL and PHBV film were collected for analysis. The water contact angles were characterized by a water contact angle instrument.

Cell Proliferation on the films of PLCL and PHBV

Forty microliter of the polymer solution were dispensed into a 96 well culture plate and placed at room temperature for 24h. The films were sterilized with 75% (V/V) ethanol solution for 2 h followed by ultraviolet radiation for 30 minutes. All the films were pre-wet with DMEM for 24 h before cell seeding. Thirty microliters of cell suspension ($1*10^5$ cells/ml) were loaded drop-wise onto the top of the films and incubated at 37°C. MTT and Hoechst 33258 were used to evaluate chondrocyte activity, attachment, and proliferation.

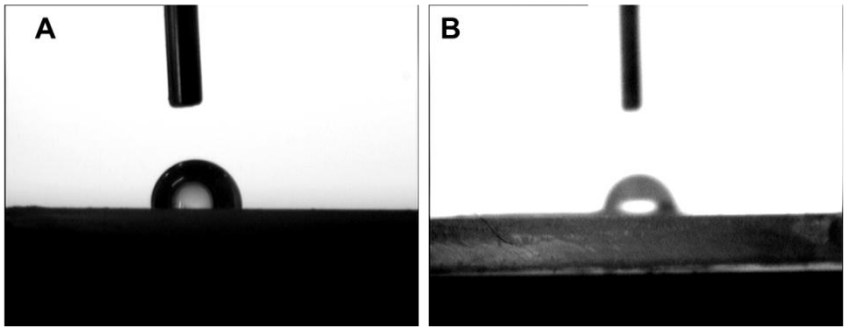
Semi-quantitative analysis

Cells viability in vitro at 6 h and 2 w were counted by 100 cells in acquired confocal figures. Cells distribution in the scaffolds was assessed by Image-Pro Plus software. If there were no more than 5% of the cells which clustered together, we could consider the cells were distributed homogeneously. Quantification of GAG and type II collagen content was performed by IPP software with the assumption that

the concentration of GAG and type II collagen was proportional to the mean optical density. GAG staining and type II collagen were assessed by IPP software around the PHBV microspheres (within 5μm) and the peripheral area（more than 5μm).

Degradation of composite scaffolds:

Weighed PLCL scaffolds and 40%PHBV/PLCL composite scaffolds were imersed into PBS(phosphate buffer solution, pH 7.4) at 37°C for 1 month and 2 months. After 1 month or 2 months, PLCL scaffolds and 40%PHBV/PLCL composite scaffolds were lyophilized for 24h and the dry weight of scaffolds were weighed again.



A: PLCL B: PHBV

	PLCL	PHBV
Contact angle θ	87.2±2.8	88.4±5.1

Fig. S1. Water contact angles of PLCL and PHBV films (n=5; P>0.05)

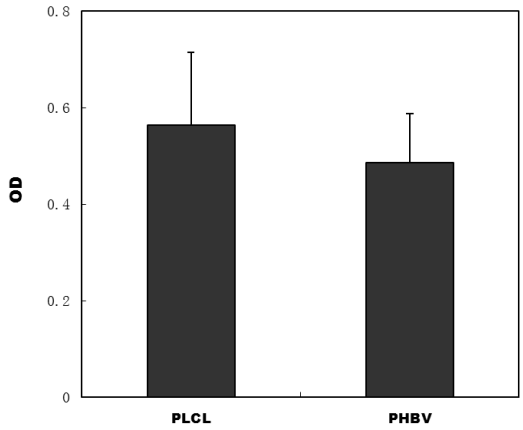


Fig. S2. Cell activities between PLCL and PHBV films at 7 days (n=5; P>0.05)

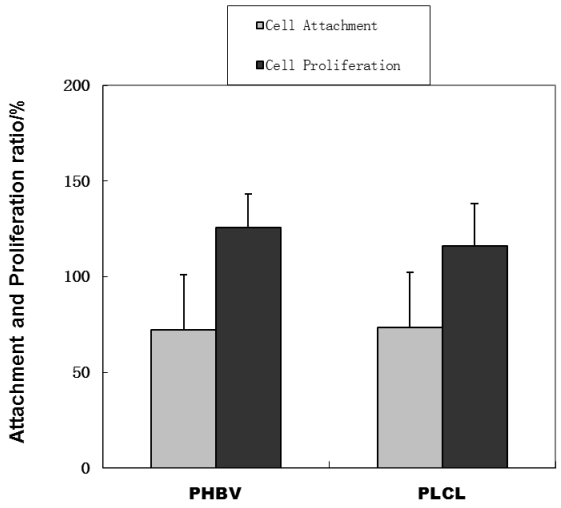


Fig. S3. Cell attachment and proliferation between PLCL and PHBV films at 6 hours and 7 days (n=5; P>0.05)

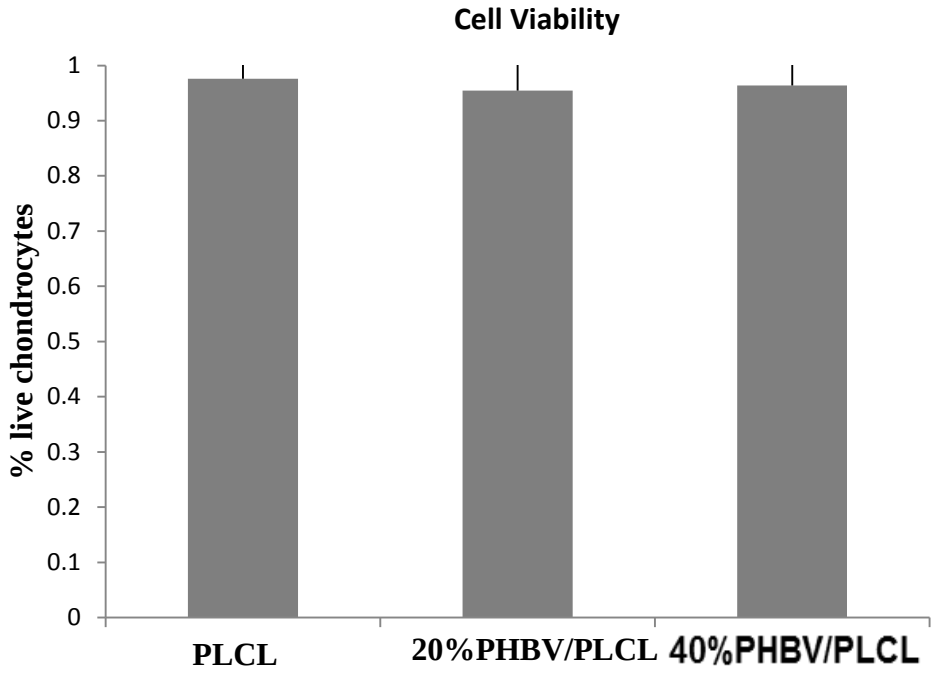


Fig. S4. Cell viability in PLCL, 20%PHBV/PLCL and 40%PHBV/PLCL scaffolds (n=5; P>0.05)

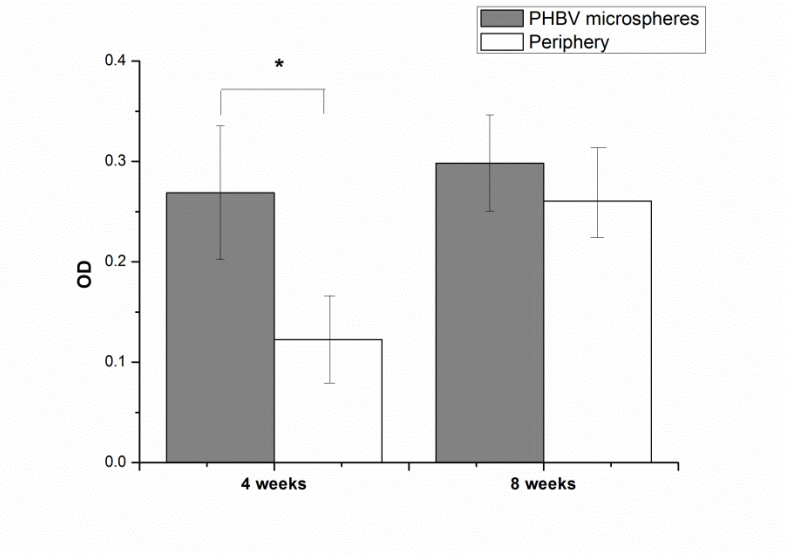


Fig. S5. GAG content on PHBV microspheres and peripheral area of PHBV microspheres after 4 weeks and 8 weeks (n=5; *P<0.05)

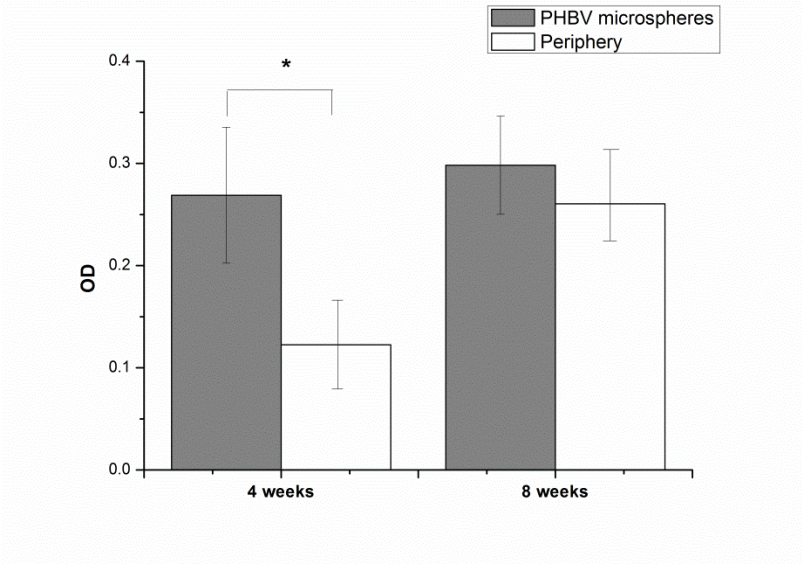


Fig. S6. Type II collagen content on PHBV microspheres and peripheral area of PHBV microspheres after 4 weeks and 8 weeks (n=5; *P<0.05)

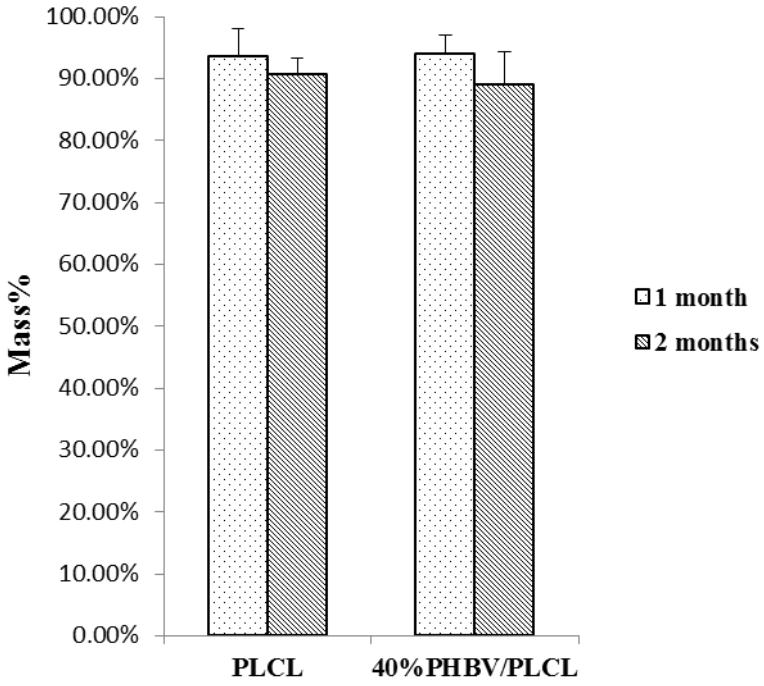


Fig. S7. Remaining mass of original PLCL scaffolds and 40%PHBV/PLCL scaffolds following in vitro degradation (n=3; P>0.05)