Electronic Supplementary Information

Highly aligned core-shell structured nanofibers for promoting phenotypic expression of vSMCs for vascular regeneration

Huihua Yuan,† Jinbao Qin,‡ Jing Xie,‡ Biyun Li,‡ Zhepao Yu,‡ Zhiyou Peng,§ Bingcheng Yi,‡
Xiangxin Lou,‡ Xinwu Lu,§ and Yanzhong Zhang,‡,§,*

† These authors contributed equally to this work

*Author to whom correspondence should be addressed.

Yanzhong Zhang, Ph.D., Tel/Fax: +86 21 6779 2374, Email: yzzhang@dhu.edu.cn
Xinwu Lu, Ph.D., Tel: +86 21 2327 1699, Email: luxinwu@shsmu.edu.cn
To demonstrate stability of the crosslinked HA/PLLA nanofibers in aqueous environment, chemistry of the nanofibrous HA/PLLA mats (crosslinked and non-crosslinked) at predetermined soaking time was examined by ATR-FTIR.

FTIR results showed that the characteristic amide peaks for HA (around 1630 cm\(^{-1}\)) in the crosslinked HA/PLLA nanofibers clearly appeared but gradually decreased with increasing soaking time. This indicated that HA layer was still retained in the crosslinked HA/PLLA nanofibers and gradually diminished during the 21 days of immersion (Figure S1B). In contrast, the characteristic amide peaks of HA disappeared in the non-crosslinked HA/PLLA nanofibers for all soaking times (Figure S1A), which suggested that HA layer was completely dissolved for only 1 day soaking. This demonstrated that stability of the HA coating layer with the HA/PLLA nanofibers can by considerably enhanced by crosslinking treatment.

![FTIR spectra of HA/PLLA nanofibers (non-crosslinked and crosslinked) after subjecting to soaking treatment in PBS for 3 weeks](image)

**Fig. S1** FTIR spectra of HA/PLLA nanofibers (non-crosslinked and crosslinked) after subjecting to soaking treatment in PBS for 3 weeks

To determine in vivo biodegradation and biocompatibility of the core-shell structured HA/PLLA nanofibers, weight loss and hematoxylin and eosin (H&E) assay were performed. Individually
weighed nanofibrous HA/PLLA and PLLA mats with the size of $1 \times 1\ cm^2$ were implanted subcutaneously in the back of SD rats (200-250 g, Shanghai Laboratory Animal Center, Shanghai). Each rat was used for subcutaneous implantation of the nanofibrous HA/PLLA or PLLA mats with four implants per time point. At day 7, 14, and 21 after the implantation, one rat implanted with HA/PLLA or PLLA nanofibers was sacrificed and the explanted samples were dried and then weighed. The extent of *in vivo* biodegradation was expressed as a percentage of the weight loss after mat implantation. The value at each time point was calculated as the mean value of the four implants for nanofibrous HA/PLLA or PLLA mats.

For histological evaluation, one rat from each sample was sacrificed after 21 days of implantation, and the surrounding tissues were excised together with the implanted nanofiber mats and fixed with 2.5% glutaraldehyde in PBS (pH 7.4). A small piece of the tissue was then embedded in paraffin before subjecting it to microtome sectioning. H&E were used for staining the tissues. The tissue response to nanofiber mats was evaluated from the coloration observed with a microscope (Nikon Eclipse 80i, Japan).

The *in vivo* weight loss for the nanofibrous HA/PLLA and PLLA mats is depicted in Figure S2A. With time, both the nanofibrous HA/PLLA and PLLA mats implanted in rats were degraded (and/or dissolved), but the HA/PLLA mats were degraded significantly faster than PLLA mats. HA/PLLA mats lost $32.99 \pm 2.20\%$ of their initial weight after 21 days compared to a $21.14 \pm 2.20\%$ weight loss for PLLA mats (Figure S2A).

Histological observation of the nanofiber mats was performed to investigate the degree of inflammatory reaction and penetration of the surrounding tissues into the nanofiber mats. Figure S2B and C show the phase contrast images of ultrathin sections of the explanted nanofiber mats stained by H&E. The nuclei of inflammatory cells were stained blue by the hematoxylin dye to show the tissue response toward the implanted nanofiber mats. As indicated by the arrows and lines in Figure S2B, a thick layer of inflammatory cells was accumulated at the interface between the PLLA nanofiber mat and the surrounding tissues. In contrast, the layer of accumulated inflammatory cells was thinner for the HA/PLLA nanofiber mat, as shown in Figure S2C. This
indicates that the HA/PLLA nanofiber mat causes smaller degree of inflammatory reaction than the PLLA nanofiber mat, likely due to the involvement of HA in the core-shell structured nanofibers.

Furthermore, delamination occurred on the surface of the HA/PLLA nanofiber mat, and hence, the infiltration of the surrounding tissues was observed. However, few infiltrations of the surrounding tissues were observed for the PLLA nanofiber mat. After 21 days of implantation, while the HA/PLLA nanofiber mat was significantly fragmented, the PLLA nanofibers retained the fiber-like morphology.

![Graph](image)

**Fig.S2** *In vivo* weight loss of the nanofibrous HA/PLLA and PLLA mats at different intervals of post-implantation (A), and histological images of the nanofibrous PLLA (B) and HA/PLLA (C) mats after 21 days of implantation.

ST: surrounding tissues; N: nanofiber mats. Scale bars = 50 μm
To show the structure and fiber orientation of the graft, the graft was cut in longitudinal and horizontal directions and observed accordingly via SEM (Figure S3), showing circumferentially aligned nanofibers that mimic the tunica media topography of the native arterial vessels.

**Fig.S3** SEM images of the cut graft in horizontal (A) and longitudinal (B) directions. Arrows in the conduits indicate viewing directions (after cutting) in SEM imaging.

Scale bars =10 μm

To view the Figure 12A and B clearly, the two immunofluorescence images were magnified and provided as Figure S4 to clarify our claim in the text. That is, ECs almost completely covered the inner lumen of the HA/PLLA grafts; in contrast, only a small area of the lumenal surface in the PLLA grafts was covered by ECs.
Fig.S4 Magnified immunofluorescence staining images of the explant cross-sections with α-SMA (red) and CD31 antibodies (green), respectively. Scale bars =400 μm