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Supplementary Information

Biocompatibility and anti-calcification research of a biological artery immobilized with a natural-occurred functionalized crosslinking reagent (phytic acid)

Supplementary Results Supplementary Figure 1 Masson staining of fresh tissue and various concentration of PA-fixed tissues Supplementary Figure 2 Photomicrographs (a) stained with Hematoxylin and Eosin & (b) stained with Masson of various tissues before and after enzymatic degradation. Supplementary Figure 3 The ultimate tensile stress of various tissues before and after enzymatic degradation Supplementary Figure 4 Photomicrographs of HUVECs cultured adjacent to tissues fixed by various concentration of PA

Supplementary Results

Supplementary Figure 1



Collagen fibers were the main component of natural extracellular matrices. To observe total framework of it inside, crosslinked arteries were stained with Masson staining and observed with $200 \times$ magnification under light microscope. As exhibited, the ultrastructure of various concentration of PA fixed tissues were all preserved well, and their ultrastructure were similar to that of fresh tissues. It indicated that PA's fixation protected properties of biological tissues well, which was also suitable for cells adhesion and proliferation.

Supplementary Figure 2



Chemical fixation improve tissues with another important physical and chemical property: its modification protect them from immediate degradation in a period of time. HE staining mainly presented the total framework and residual nucleus of examined biological arteries. As seen in SIFig. 2(a), after 24h collagenase degradation, it was obvious that the fiber structure in fresh tissues turned sparse and finer, even become chaotic and disintegrated into pieces, which implied extensive degradation effect. In contrast, microstructure of the other three crosslinked samples were all preserved well and remained intact.

Since collagen fibers were the main component of natural extracellular matrices, Masson staining was employed to specially characterize the arrangement and distribution of collagen fibers inside. In SIFig. 2(b), similar phenomenon was exhibited: After crosslinking treatment, the collagen fibers were all remained intact. However, those in fresh tissues after collagenase degradation were degraded extensively. This result was in accordance with previous observation on HE staining.

Supplementary Figure 3



SIFig. 3 presented the ultimate tensile stress before and after collagenase degradation of all tested samples. As illustrated, the ultimate tensile stress of crosslinked tissues increased significantly, while the value for fresh tissues after degradation was extremely low and hard to determine for complete degradation. Moreover, among three fixed samples, the decline in mechanical strength for 5% PA-fixed tissues was more evident than GA-fixed one, which indicated stronger resistance against enzymatic degradation for GA fixed tissues. Therefore, connected with previous result obtained in weight loss measurement during degradation process in Fig. 6, it was notable that 5% PA-fixed arteries possessed ideal resistance against enzymatic degradation in early stage and some biodegradability in longer period, which was important for crosslinking reagent to biological originated engineering scaffolds.

Supplementary Figure 4



SFig. 4 presented the growth of HUVECs cultured in the vicinity of the tissues fixed by different concentration of PA during culture period. As shown, a distinct increasement in cellular quantity was observed surrounding tissues with the concentration of PA gradient raised from 0.5% to 5%, which indicated lower cytotoxicity of these samples. However, a distinct decrease in cellular quantity occurred when tissues fixed by PA with concentration 7.5% and 10%. It implied that, among these measured PA-fixed tissues, the samples fixed by 0.5%, 1%, 2% and 5% PA possessed better cytocompatibility. With its concentration further increased to 7.5% and 10%, a distinct decrease in cellular quantity and some distorted cells were observed adjacent to tissues, which represented higher cytotoxicity. This result was in agreement with CCK-8 assay of extraction shown in Fig. 7.