A Novel Single-species Self-assembly Process to Fabricate Collagen-like Nano-fibrous Poly(L-lactic acid) Scaffolds

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Introduction

While a variety of surface modification methods have been developed to fabricate biomimetic scaffolds, it is still a challenge to achieve a uniform distribution of the modification agent throughout the 3D scaffold with complex geometry. Electrostatic layer-by-layer self-assembly (ESA) technique is a novel and promising technique to prepare well-defined surfaces. Traditionally, the ESA technique involves two different species (polyanion and polycation), which inevitably incorporates a non-modification agent onto to the scaffolds. This non-modification agent often does not have good biocompatibility or biodegradability. In this study, we developed a novel single-species ESA process to prepare collagen-like nano-fibrous PLLA (NF-PLLA) scaffolds. Gelatin, a collagen-derived species with two different types (type A and type B), was utilized to modify the surface of NF-PLLA scaffolds via the ESA technique. This single-species ESA process for surface modification eliminates the potential side effects of adding a second species. The gelatin-modified NF-PLLA scaffolds, which mimic both the physical architecture and chemical composition of natural collagen, were examined in this study for cell adhesion and proliferation.

Methods

NF-PLLA scaffolds were fabricated using a thermally induced phase separation and porogen leaching method. The pretreated NF-PLLA scaffolds were first immersing in gelatin type A (GA) solution (pH=6.6). After washing of the scaffolds with water, the scaffolds were dipped into gelatin type B (GB) solution (pH=6.4) and then washed with water. The further growth of GA/GB bilayers was accomplished by the repetition of the same cycle of immersion of GA solution, rinsing, immersion into GB solution, and rinsing. The GA/GB architecture was fixed by crosslinking gelatin with EDC and NHS in MES buffer. The surface-modified scaffolds were obtained by freeze-drying.

Results

Confocal images indicated that the deposited gelatin was distributed evenly throughout the entire surface (both outer and inner surfaces) of the NF-PLLA scaffolds (Figure 1). The surface coverage of gelatin on the NF-PLLA surface was controlled by the assembled polyelectrolytes bilayer numbers, and increased linearly with the bilayer numbers ($n \geq 1$). The contact angles decreased after the NF-PLLA was deposited with gelatin, indicating the increasing of hydrophilicity of the surface-modified NF-PLLA. Human dental pulp stem cells were cultured on both surface-modified NF-PLLA scaffolds and NF-PLLA controls. The number of cells on the surface-modified NF-PLLA scaffolds was significantly higher than that on the controls 24 h after cell seeding. Histology images showed that the surface-modified NF-PLLA scaffolds contained more cells, and the cells were more evenly distributed throughout the center of the scaffolds compared to the NF-PLLA controls after 1 and 2 weeks of cell seeding. These results demonstrated that the single-species ESA process was an effective strategy to modify the three-dimensional scaffold surfaces, and the gelatin-modified nano-fibrous architecture could serves as a superior scaffold for tissue engineering.

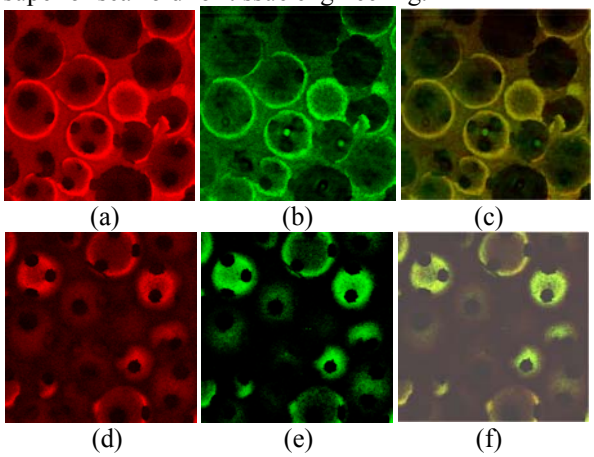

Figure 1. Confocal images of NF-PLLA scaffolds after surface modified with TRITC-conjugated GA and FITC-conjugated GB. (a-c) the surface of the 3D scaffold, (a) showed TRITC-conjugated GA, (b) showed FITC-conjugated GB, while (c) was the overlap of (a) and (b); (d-f) the center of the 3D scaffold, (d) showed TRITC-conjugated GA, (e) showed FITC-conjugated GB, (f) was the overlap of (d) and (e).

Conclusions

In this study, we developed a novel single-species ESA process to modify the surface of NF-PLLA scaffolds. Gelatin, a collagen-derived species with two different types (type A and type B), was successfully incorporated onto the surface of NF-PLLA scaffolds during the ESA process. The deposited gelatin was observed to distribute evenly throughout the entire scaffold surfaces. Control over the amount of deposited gelatin on the NF-PLLA scaffolds was achieved by varying the self-assembled bilayer number. The gelatin-modified NF-PLLA scaffolds significantly enhanced cell adhesion and proliferation.

References

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