Fabrication of Cross-linked Gelatin Microfibers by Photochemical Reactive Electrospinning

Jian-Feng Zhang, Sumei Zhong, Thomas E. Lallier, Xiaoming Xu
Louisiana State University Health Sciences Center, School of Dentistry, New Orleans, USA.

Statement of Purpose: Gelatin is a denatured protein derived from partial hydrolysis of collagens and has been widely used in pharmaceutical and medical fields as sealants for vascular prostheses, drug delivery carrier, etc, due to its biodegradability, biocompatibility, and relatively low cost [1,2]. Gelatin nano/micro-fibers can be electrospun from highly toxic fluorinated alcohol, but gelatin fibers are very fragile, water-soluble, and cannot be used as a scaffold material for tissue engineering. To improve their water-resistance and mechanical strength, cross-linking, mainly by toxic cross-linkers such as glutaraldehyde, is usually required. Therefore, less toxic or non-toxic methods for fabrication of cross-linked gelatin fibers are needed. The objective of this pilot study is to fabricate cross-linked gelatin micro-fibers using a novel reactive electrospinning method from a non-toxic solvent (mixture of PBS and ethanol) and *in situ* photo-cross-linking or its combination with genipin, which has low cytotoxicity. The biocompatibility and cell attachment to these gelatin microfibers were tested using periodontal ligment (PDL) cells.

Methods: Methacylated gelatin was synthesized from type A porcine skin gelatin (10%) and methacrylate anhydride (MA) in DPBS at 50 °C. The mixture was dialyzed with water for 1 week at 40 °C to remove salts and resultant methacrylic acid. The solution was further lyophilized for 1 week. Gelatin with two different methacrylation degrees (low and high, as Group 1 and 2) was prepared. The methacrylated gelatin was dissolved in PBS/ethanol (1:1) and 1% of photoinitiator was also added. In Group 3, 0.1% genipin was added to the methacrylated gelatin with high methacrylation degree and stirred for 2 hours. The gelatin microfibers were fabricated using the photochemical reactive electrospinning method as previously reported [3,4]. Tissue culture plastic was used as (positive) control (Group 4). PDL fibroblasts were isolated from extracted teeth and maintained in Minimal essential media containing 10% fetal bovine serum. 50,000 cells were added to each microfiber sample in a volume of 50 µL and allowed to adhere for 24 hours. Cell survival was assayed fluorometrically using Calcein-AM.

Results: Methcrylated gelatin at concentrations between 80 – 125 mg/mL in non-toxic PBS/ethanol (1:1) solution could be electrospun into microfibers. Photo-cross-linked gelatin microfibers showed swelling in PBS/ethanol (1:1) but not dissolved after 28 hours immersion. Uncross-linked microfibers electrospun from raw and methacrylated gelatin rapidly dissolved in PBS/ethanol (1:1). Compared to control, the methacrylated gelatin with low methacrylation degree had 77% PDL cell attachment and survival (Group 1). Higher methacrylation degree reduced cell attachment and survival rate to 49% (Group 2). With 0.1% genipin and higher methacrylation degree, cell attachment and survival rate was 61%. PDL cells cultured on Group 1, 2 and 4 had the long spindle shape. However, the dual cross-linking gelatin had shortened, and more round cells, suggesting more cell death or poor cell attachment.

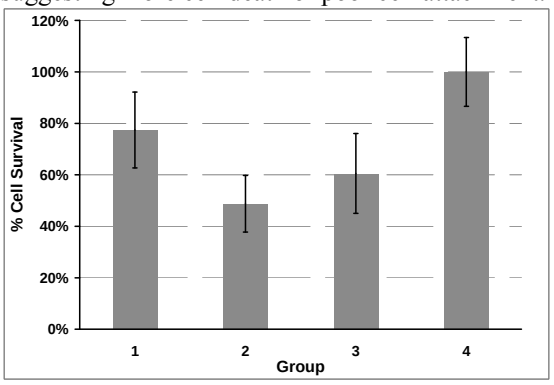

Figure 1. Relative PDL cell survival rates on cross-linked gelatin microfibers and control. Group 1: photo-cross-linked gelatin with low methacrylation degree; Group 2: photo-cross-linked gelatin with high methacrylatin degree; Group 3: Dual cross-linked gelatin fibers by 0.1% genipin and high methacrylation degree; Group 4: Tissue culture plastic as positive control.

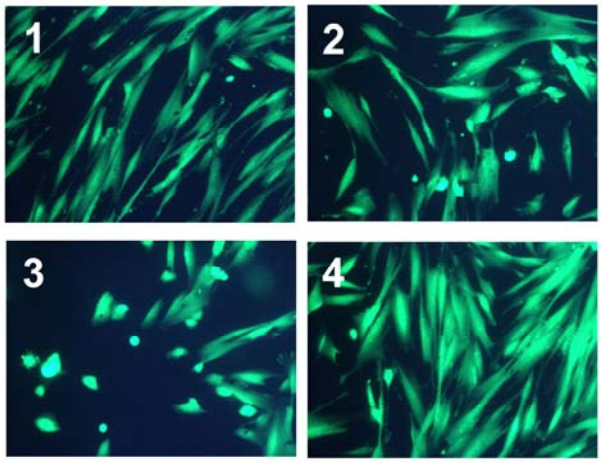

Figure 2. Fluorescence pictures of live cells on cross-linked gelatin microfibers and tissue culture plastic.

Conclusions: Photo-cross-linking provides less toxic route to prepare water insoluble gelatin micro-fibers for tissue engineering applications. Methacrylation degree of gelatin should be optimized to reduced potential cytotoxicity.

References:

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