Enhancing cell behavior on 3D scaffolds by plasma-based 3D printing system

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Introduction and Statement of Purpose:

Melt extrusion additive manufacturing (ME-AM) also known as the fused deposition modeling (FDM) is the most generally used at 3D printing techniques which is widely used to fabricate structures mimicking the extracellular matrix for tissue regeneration. Among used various materials, poly lactic acid (PLA) is a well-known biomedical polymer with biodegradable and mechanical properties . Despite, it has a limitation such as low wettability and shows poor cell attachment, because of its low surface energy. Plasma treatment is used to render the surface modification to these drawbacks. Generally, plasma treatment is performed post-process, so inhomogeneous and impossible to uniform surface modification on inner part. To overcome this limitation, we converted one of two nozzles in an FDM type 3D printer to a plasma device to process each inner layer.

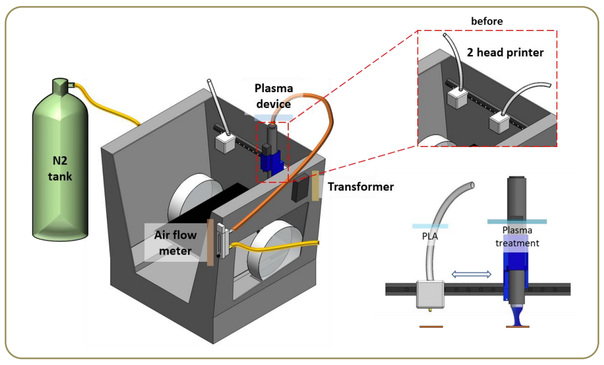

Figure 1. Schematic illustration of the plasma-based 3D printing system.

Methods, materials and analytical procedures:

The extruder component was changed from the one of two head ME-AM type 3D printer (BCN 3D Sigma R19) to a plasma device (Piezobrush PZ2-i) for treating plasma. The treated plasma volume is controlled by G-code regulating plasma device movement. The samples were prepared with disc type scaffolds (9 mm in diameter, 6 mm height) and stored at room temperature in sealed container for overnight. SEM, non-contact surface profiler, and contact angle, were utilized to observe surface characterization of the plasma own effect. In addition to evaluate the in vitro biocompatibility of plasma-treated scaffolds which were treated 70 mm/s, 20 mm/s, and 7 mm/s of treated-speeds, green fluorescent protein-expressing human-derived mesenchymal cells (GFP-MSC) were seeded on the top of scaffolds. Subsequently, layer-by-layer treated (LBLT) scaffold (10 mm x 10 mm x 3.5 mm) was prepared for comparing with scaffold as generally plasma treatment way (GT) and placed at room temperature for overnight, washed with Dulbecco's phosphate-buffered saline (DPBS) and dried under the ultraviolet light . To assess cellular behavior of scaffolds with each plasma-treated system, fabricated spheroids were seeded on scaffolds. Additionally, scaffolds were assessed by protein absorption assay with complete medium.

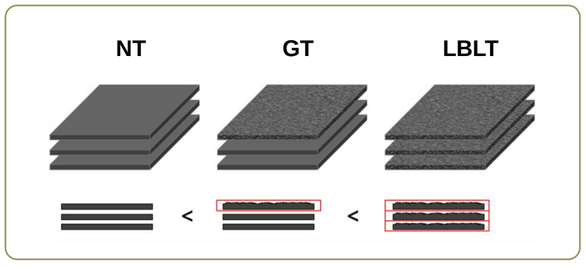

Figure 2. Schematic illustration of printing scaffolds by plasma-based 3D printing system.

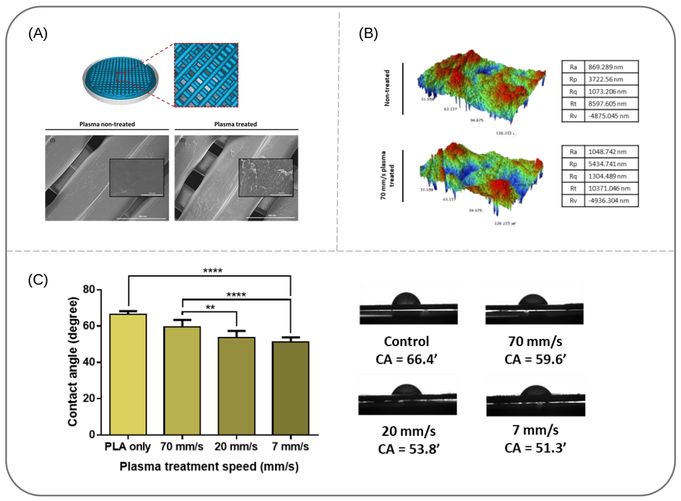

Figure 3. Evaluate of the plasma treatment effect ;(A) scanning electron micrograph (SEM) image of the plasma non-treatment PLA and plasma treatment PLA scaffold, (B) non-contact roughness measurement results, and (C) water contact angle measurement after treatment by plasma treatment at room temperature.

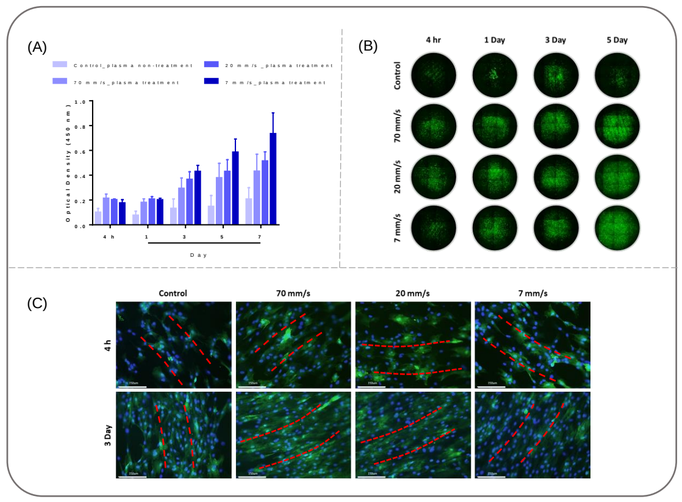

Figure 4. In vitro characterization of cell proliferation ratio of GFP-MSC for 5 days of culture. (A) cell proliferation test of plasma treated scaffolds, (B) fluorescence microscopy overview images, and (C) fluorescence images after 4 hours and 3 days of culture.

Results:

To apply the plasma-based 3D printing system, one of two nozzles in an FDM type printer converted to a plasma device (Fig 1). The G-code was revised for plasma movement and continuing stack of PLA filament using BCN3D.Cura-3.2.0-win64.

Surface characterization results for confirm of plasma treatment effect show that the plasma treated scaffolds were induced roughness and hydrophilicity (Fig 3A-C). In addition, plasma treated scaffolds show remarkable proliferation and the more plasma is treated, the better cell proliferation was increased (Fig 3D-E). Therefore, plasma treatment speed was fixed to 70 mm/s, due to a process time and consumption of gas. Then, layer-by-layer treated scaffold as 70 mm/s treated speed was fabricated to evaluate the effect of plasma treatment with non-treated scaffold (NT) and generally treated scaffold (Fig 2).

The results show that LBLT was enhanced with biocompatibility and cellular behaviors than NT. Fig 4C shows that NT remains round shape of spheroid, GT and LBLT shows loosing the round shape of spheroid. Besides, LBLT was improved migration into inner part. Also, protein absorption assay show LBLT the most increased than others. Similarly, Fig 5 indicate that SEM analysis to evaluate changes in spheroid morphology and confirm migration of cells into inner part.

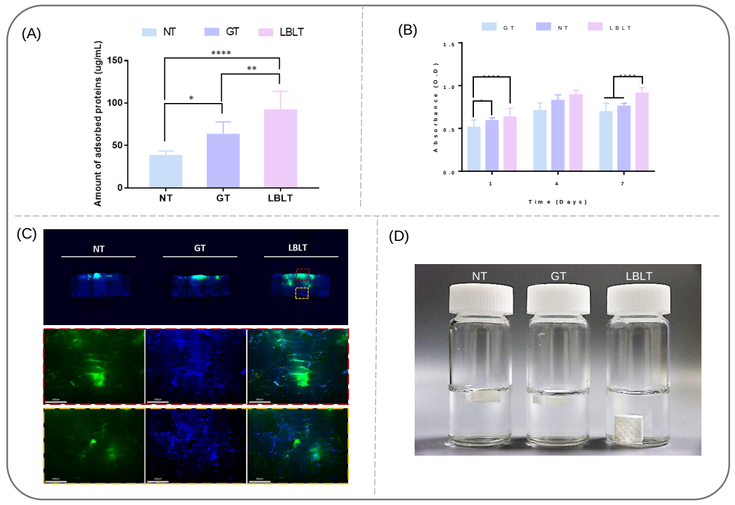

Figure 5. Demonstrate of effect of layer-by-layer treatment system ; (A) amount of adsorbed serum proteins to the scaffolds, (B) proliferation rate of GFP-MSC spheroids on scaffold by Ez-cytox, (C) representative fluorescence images of GFP-MSC spheroids on scaffold and image of cross-section after 7 days, and (D) a photograph of the immersed in PLA scaffold after 24 hours on DPBS.

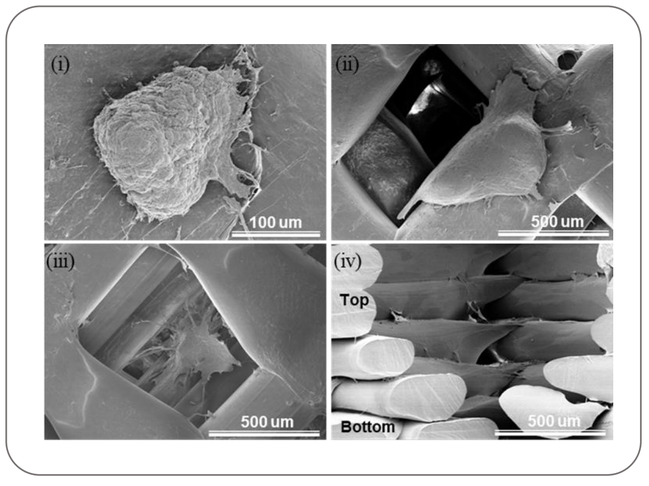

Figure 6. SEM image of GFP-MSC spheroids adhered on top layer of scaffold ;Con (i), LT (ii), LBLT (iii), and LBLT cross section (iv).

Discussion and Conclusions:

In this works, we presented a plasma-based 3D printing system for fabrication of scaffold which uniformly modified inner parts. In this regard, we demonstrated easily apply to 3D printing system and each layer plasma-treated scaffolds were enhanced biocompatibility. Our results, indicate that (1) developed of homogeneous surface modified scaffold system, (2) enhanced topography, roughness, and hydrophilicity, (3) promoted higher protein absorption, cell proliferation, and migration into scaffold inner part, and (4) easily apply to all the two head deposition system. Therefore, our system suggest that layer-by-layer treated scaffolds can fast fabricated and be a useful strategy for tissue engineering in the future.

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