Transactions on Additive Manufacturing Meets Medicine
Trans. AMMM, Vol 2, No 1, 2020, Article ID 020

Infinite Science | Publishing

Polydopamine functionalized 3D printed scaffolds for bone tissue engineering

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Abstract: 3D printing offers unique solutions for bone tissue regeneration with an opportunity to engineer scaffolds with the desired structure, shape, and porosity. Scaffold's surface-to-cell interaction is critical for better tissue integration and regeneration. To achieve this, we have surface modified the 3D printed scaffolds with polydopamine (PDA) to evaluate the potential of the surface modification for bone regeneration.

I. Introduction

Bone regeneration is a complex and precise process regulated by a large number of factors like osteocytes, osteoclasts and endothelial cells [1]. Surface properties of scaffolds are one of the critical factors that will influence cell attachment, proliferation, and differentiation of stem cells towards osteoblasts [2]. The surface chemistry of the implant is crucial for better interaction of the material with the surrounding tissue. Three dimensional (3D) printing with the fused deposition modeling (FDM) method is one of the more widely explored 3D printing approaches that works by extruding semi-molten polymer from a heated nozzle onto a platform in a layer-by-layer fashion [3-6]. Polylactic acid (PLA) is a biocompatible and biodegradable polymer that is suitable for fabricating scaffolds with defined structure and strength [5]. For the 3D printing carried out at higher temperatures, surface functionalization is vital to impart osteogenic properties to 3D printed scaffolds without disturbing their structure and strength. PDA is an amine rich self-assembled polymer which has superior adhesive properties towards bioactive components (such as DNA and proteins) and was reported to have osteogenic properties itself [7, 8]. 3D printed scaffolds have specific pore size and architecture while other casting techniques deliver random pore size. Moreover, the technique is facile and easy to generate similar structures repeatedly without batch-to-batch variation. Hence in this study, we developed and evaluated the osteogenic properties of PDA surface-functionalized 3D-printed scaffolds for bone tissue regeneration (Figure 1).

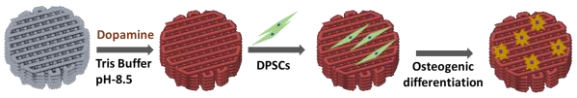

Figure 1: Cartoon depicting the procedure of PDA coating the 3D printed PLA scaffolds and it's in vitro interaction with DPSC cells.

II. Materials and methods

PLA scaffolds of 6.22 x 2 mm were 3D printed using Prusa i3MK3 MMU2.0 (Prusa Research, Czech Republic), with infill densities of 70 % at 150 mm/s print speed. The computer-aided design files were created using Fusion360 software and exported as .stl files. The .stl files were processed using Slic3r Prusa edition and exported as a .gcode file.

The PDA surface functionalization was carried out by incubating the PLA scaffolds in dopamine solution (10 mM Tris, pH-8.0) for 30 min to 24 h. The dopamine self-assembles to a polymer form on the scaffold surface (PLA-PDA) [8]. The scaffolds were characterized using scanning electron microscopy (SEM). The wettability determination was studied using a contact angle goniometer. The scaffolds were washed with PBS and used for *in vitro* studies.

The osteogenic ability of the scaffolds was studied using dental pulp stem cells (DPSC, Lonza) and the osteogenic differentiation of the DPSCs towards osteoblasts was measured using RT-PCR on day 7 and Alizarin red staining on day 14. The RNA was extracted using the Rneasy mini kit (Sigma, USA) and converted to cDNA (Applied Biosystems, USA). The primers for osteocalcin, alkaline phosphatase, and Runx-2 were procured from IDT technologies, USA. The RT-PCR was performed using the SYBR fast method, and the data was analyzed by $2^{-\Delta\Delta Ct}$ method. The alizarin red staining was performed by fixing the scaffolds with 3.7% formaldehyde for 30 min and then the samples were stained with 2% alizarin red stain for 30 min. The scaffolds were washed and the dye was dissolved in 1 N HCl for 12 h, and read at 410 nm (SpectramaxM5, USA)

III. Results and discussion

Figure 2 shows the surface morphology of the 3D printed PLA and PLA-PDA scaffolds. The SEM image of the PLA

DOI: 10.18416/AMMM.2020.2009020

showed a smooth surface morphology. The PDA functionalization at 10, 30 min showed a smooth surface while functionalization at 2 and 24 h showed a rough surface with white PDA particles [9]. The scaffolds color (insets of figure 1) also changed from white to brown, indicating the PDA functionalization of PLA scaffolds.

Figure 2: SEM images of [A] PLA and [B-E] PLA scaffolds incubated with PDA for 10 min, 30 min, 2 h and 24 h respectively. Insets show the optical images of the scaffolds

Figure 3 shows the change in the wettability of PLA with PDA surface functionalization. The PLA scaffold showed a contact angle of 87±1.6 while the contact angle for PLA-PDA-10 min, 30 min, 2 h, and 24 h is 47±1.0, 20±1.4, 6±0.4 and 26±3.9 respectively. The contact angle gradually decreased with time except for 24 h, which might be due to the prolonged incubation and surface roughness. However, PLA-PDA-24 h showed significantly higher wettability compared to PLA. The PLA-PDA 2h showed higher wettability, which helps for better cell attachment, and hence this group was used for *in vitro* studies.

Figure 3: Contact angle of [A] PLA and [B-E] PLA scaffolds incubated with PDA for 10 min, 30 min, 2 h and 24 h respectively.

Figure 4A & B-C shows the cell proliferation and cell attachment of DPSCs on PLA, and PLA-PDA. Cell proliferation increased from day 3 to day 14 across all the groups. The SEM images in Figure 4B showed strong surface-to-cell interaction with high surface coverage in PLA and PLA-PDA scaffolds.

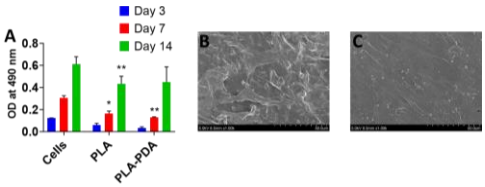

Figure 4: [A] Cell proliferation of DPSCs. The samples were run n=3, and the data was represented in mean±SD with p<0.05 (ANOVA). [B&C] cell attachment of DPSCs on PLA, and PLA-PDA scaffolds at day 14.

The expression of osteogenic genes which were evaluated with RT-PCR is shown in figure 5. Osteocalcin, Alkaline phosphatase, and Runx-2 genes are known to be differentiation and maturation markers of osteoblastic lineage. The PLA-PDA showed significant expression of these genes compared to PLA[10]. Alizarin red staining quantification showed significant increases in mineralization in the PLA-PDA group compared to cells alone [11].

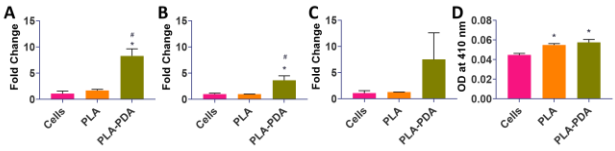

*Figure 5: RT-PCR of [A] Runx-2, [B] Alkaline phosphatase, [C] Osteocalcin genes and [D] Quantitative analysis of the alizarin red staining of calcium mineralization on scaffolds. Significant difference indications imply a difference from the groups Cells (*), PLA (#), PLA-PDA (^). The data was represented as mean±SD with *p<0.05 and n=3.*

Our results suggest that PDA surface functionalization is a promising strategy and warrants *in vivo* testing for bone regeneration applications. It would also be interesting to evaluate the bone regeneration efficacy of this novel modified scaffold when it is synthesized to deliver bioactive molecules such as growth factors or genes.

IV. Conclusions

PLA-PDA scaffolds showed increased wettability, enhanced cell proliferation, and osteogenic potential with increased expression of osteocalcin, Runx-2, alkaline phosphatase genes, and increased mineralization.

Acknowledgments

The authors acknowledge the Central Microscopy Facility, University of Iowa. The authors thank Zaid, Assaf from Wurster's lab, College of pharmacy for his help in contact angle measurement. Research funding: The Lyle and Sharon Bighley Chair of Pharmaceutical Sciences.

Author's Statement

Conflict of interest: Authors state no conflict of interest.

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