

RESEARCH ARTICLE

The mussel-inspired assisted apatite mineralized on PolyJet material for artificial bone scaffold

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Abstract: With the development of three-dimensional (3D) printing, many commercial 3D printing materials have been applied in the fields of biomedicine and medical. MED610 is a clear, biocompatible PolyJet material that is medically certified for bodily contact. In this study, the polydopamine (PDA)/hydroxyapatite (HA) coating was added to the printed MED610 objects to evaluate its physical properties, cell proliferation, cell morphology, and alkaline phosphatase expression level. The results show that the PDA/HA coating helps printed objects to enhance the hardness, biocompatibility, and osteogenic differentiation potential. We expect that PDA/HA coatings contribute to the applicability of MED610 in biomedical and medical applications.

Keywords: Three-dimensional printing; Polydopamine; Hydroxyapatite; MED610; Osteogenic differentiation; PolyJet technology

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Received: April 10, 2019; **Accepted:** May 27, 2019; **Published Online:** July 11, 2019

Citation: Chen Y, Fang H, Shie M, *et al.*, 2019, The mussel-inspired assisted apatite mineralized on PolyJet material for artificial bone scaffold. *Int J Bioprint*, 5(2): 197. <http://dx.doi.org/10.18063/ijb.v5i2.197>

1. Introduction

With the development of multidetector computed tomography and magnetic resonance imaging (MRI), the three-dimensional (3D) structure of the patient's body can be presented by these medical imaging techniques and computer-aided software engineering. The 3D structures can be converted to the interpreted surface tessellation language files for 3D printing by the computer software processing. Therefore, the 3D printing technology in modern medicine has great development in current years^[1]. The events of 3D printing applied in current medical practice were shown below: (1) The manufacturing of *in vitro* mechanical aids: The 3D printing technology

can be used to create customized and mechanical aids to restore patient's physical control and action. Cases: Arthrogyrosis multiplex congenital and paralyzed patients^[2]. Advantages: The small number of customized products can be manufactured. (2) The development of the customized implant molds: The required and customized metal or silicone implants can be fabricated through the molds which are obtained by the 3D printed patient's desired polymer implant models^[3,4]. (3) The pre-operative 3D model: By 3D printing of patient's tissue models, the more complicated or high-risk surgery can be planned in advance and the time of surgery can be shortened to decrease the surgical risk^[4]. In addition, 3D printing model can also facilitate the improvement of commercial

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products, such as artificial pelvis^[5]. (4) The manufacturing of customized implants: The implants were fabricated by 3D printing technology and can be used for long-term implantation. Cases: 3D printed bioresorbable airway splint for tracheobronchomalacia^[6], 3D printed skull implant^[7], hip^[8], pelvis^[9], jaw^[10] and so on.

Although 3D printing in modern medicine had many practical cases and has a lot of advantages, it still has many challenges that need to be solved. First, the biocompatible materials for 3D printing in the market are limited and the materials cannot satisfy the specific needs of desired strength, flexibility, and hardness. In addition, the polymerization of 3D printable materials usually involved with hot and organic solvents. The materials did not have good biocompatibility and cannot be used in biomedical and tissue engineering. Although some natural materials such as collagen or gelatin have been applied in 3D printing technology^[11], they cannot provide good mechanical properties and the strength are usually increased by toxic cross-linking agents. Therefore, developing new biocompatible materials or new strategies to enhance the biocompatibility of printed objects is a very important issue.

In the current year, scientists have devoted considerable attention to polydopamine (PDA)-related research^[12]. PDA has the excellent adhesive force to bind with various substrates containing plastics, oxides, noble metals, ceramics and so on, and it can also supply secondary reactivity for conjugating molecules^[13-15]. It provides a new strategy through simple chemistry to modify various substrates and increase the function and biocompatibility of substrates^[16-18]. In 2014, Wu's group showed self-assembled Ca-P/PDA composite nanolayers can modify the surface of materials and provide the bioactivity for bone regeneration^[19]. In our previous study, we also demonstrated the angiogenesis and osteogenesis of human mesenchymal stem cells (hMSCs) cultured on the polycaprolactone scaffold with PDA-coated/hydroxyapatite (HA) precipitate can be promoted^[20].

In this study, PDA-coated/HA precipitate was modified on the objects printed with the commercial PolyJet photopolymers (MED610), which only support short-term mucosal-membrane contact of up to 24 h. The modified objects had greater biocompatibility and better osteogenesis ability. These results pointed out that this strategy can increase the biocompatibility of printed objects and facilitate the development of 3D printing in biomedicine.

2. Materials and Methods

2.1 MED610 3D Printed Object Fabrication

The 3D printed objects were through SolidWorks (Dassault Systemes SolidWorks Corp., USA) and fabricated by a Stratasys Objet500 Connex3 PolyJet printer

(Objet/Stratasys, USA) with MED610 biocompatible photopolymers (Objet/Stratasys, USA). The objects were printed with a thickness of 3 mm and a diameter of 6 mm for the evaluation of mechanical properties and with a thickness of 3 mm and a diameter of 6 mm for the biological test. The uncured photopolymers were washed away, and the objects were post cured under UV light to obtain fully cured objects. In addition, the objects were washed again for cell culture.

2.2 PDA Coating and HA Mineralization

The PDA was deposited onto the MED610 3D printed objects by direct immersion coating. The objects were immersed in a solution of dopamine hydrochloride (Acros Organics) (2 mg/mL in 10 mM Tris, pH 8.5) for PDA coating, shaken at 25 rpm for 12 h at room temperature, and then rinsed several times with deionized water and dried. For HA crystal mineralization, the objects were treated with calcium and phosphate solution. 10× simulated body fluid (SBF) solution was chosen to initiate uniform nucleation and growth. A stable stock solution of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (1.198 g), NaCl (58.44 g), KCl (0.375 g), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (1.016 g), and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (3.675 g) at pH of about 4.1 was prepared and then added NaHCO_3 (0.84 g) was added to adjust the pH to about 6.3. The objects were immersed in 10×SBF at room temperature for 1 h.

2.3 Characterization

Using X-ray diffractometry (XRD; Bruker D8 SSS, Karlsruhe, Germany) at 30 kV and 30 mA with a scanning speed of 1°/min., the phase composition of the objects with or without coatings was analyzed and the concentration of the measured elements was given in atomic percent. Besides, the scanning electron microscope (SEM) images of the samples were obtained with a SEM (SEM; JSM-6700F, JEOL) operated in the lower secondary electron image mode at 3 kV accelerating voltage. Furthermore, the hardness of the samples was evaluated by the Vickers hardness test.

2.4 Cell Proliferation

All samples were immersed in 75% ethanol and exposed to UV light for 30 min for sterilization before cell experiments. The hMSCs were obtained from Sciencell Research Laboratories (Sciencell, Carlsbad, CA) and cultured with mesenchymal stem cell culture medium (Sciencell) at 37°C in a 5% CO_2 atmosphere. Cell suspensions at a density of 10^4 cells/sample were directly seeded on each sample. The cells were cultured on tissue culture plates without materials (control, Ctl) or the objects with or without coatings for different days and the cell viability was evaluated by the PrestoBlue® (Invitrogen, NY, USA) assay. The optical density was

obtained in a multi-well spectrophotometer (Hitachi, Tokyo, Japan) at 570 nm with a reference wavelength of 600 nm.

2.5 Cell Morphology

After 12 h of cell culture, the samples with hMSCs were washed with cold PBS and fixed by 1.5% glutaraldehyde (Sigma-Aldrich, MO, USA) for 2 h and then were dehydrated by a graded ethanol series for 20 min at each concentration and dried with liquid CO₂ by a critical point dryer device (LADD 28000, LADD, Williston, VT, USA). The dried samples were mounted on stubs, coated with gold particles, and investigated by SEM (JEOL JSM-7401F, Tokyo, Japan).

2.6 Osteogenesis Assay

After 3 and 7 days of cell culture, the level of alkaline phosphatase (ALP) activity was evaluated using p-nitrophenyl phosphate (pNPP, Sigma) as the substrate. The samples were mixed with pNPP in 1 M diethanolamine buffer for 15 min, then stopped by the addition of 5N NaOH and quantified by absorbance at 405 nm. The experiments were performed in triplicate.

2.7 Statistical Analysis

A one-way variance statistical analysis was used to evaluate the significance of the differences between the groups in each experiment. Scheffe's multiple comparison test was used to determine the significance of the deviations in the data for each specimen. In all cases, the results were considered statistically significant with $P < 0.05$.

3. Results

3.1 The Physical and Chemical Characterization of PDA/HA Scaffolds

Biocompatible materials for 3D printing on the market are limited and the material does not meet the specific needs of the desired hardness, strength, and flexibility. In this study, we used the commercial PolyJet photopolymers (MED610) as the test samples to investigate if PDA/HA coating can enhance the biocompatibility of printed objects and facilitate to improve the applicability of commercial materials.

Figure 1 shows the images of printed objects without and with PDA coating. The printed object coated with PDA exhibited black color and this result indicated that we have successfully modified the PDA to the surface of the object.

The XRD patterns of the MED610 object (M), the MED610 object with HA (MHA), the prepared PDA-

coated object (MP), and the PDA/HA-coated object (MPHA) are shown in Figure 2. M and MP have no peaks. The peaks of MHA and MPHA at around $2\theta = 25.7^\circ$ and $2\theta = 31.9^\circ$ are characteristic of HA precipitates, which occurs during the early mineral phase of bone development and fracture healing^[21]. The result shows the PDA/HA-coated MED610 object contains a large amount of HA precipitate.

Figure 3 shows the SEM results of MED610 object with HA, PDA, or PDA/HA coatings. The PDA/HA coated object presents more HA mineral crystallization. Based on these results, it is speculated that the PDA coating can effectively assist the bionics of HA mineralization, thereby producing a hybrid biomaterial having HA. In addition, the addition of a PDA/HA coating can increase the hardness of the printed object (Figure 4).

3.2 Cell Proliferation and Morphology

Whether the biomedical materials printed by the 3D printer can be widely used in the medical field, the

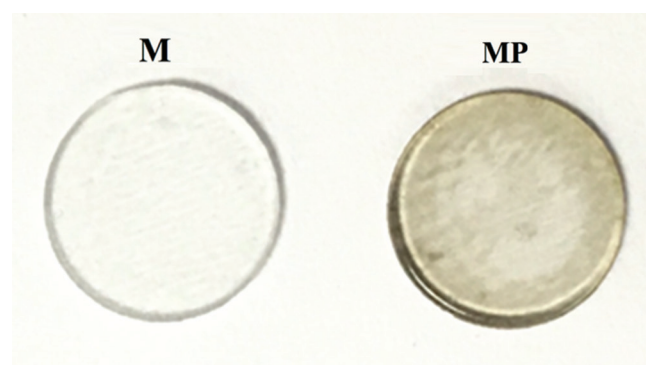


Figure 1. The images of the top view of printed objects without (M) and with (MP) PDA coating. The thickness of the objects is 3 mm and a diameter of 6 mm.

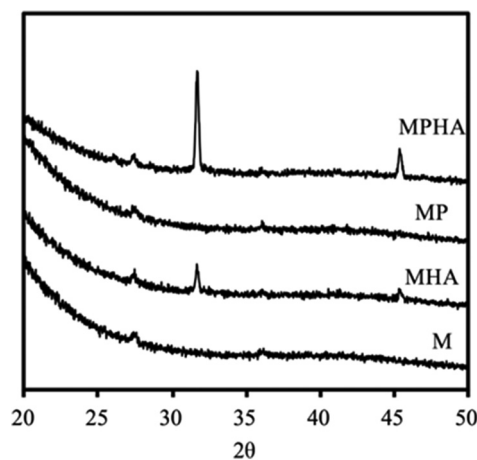


Figure 2. The wide-range X-ray diffractometry patterns of MED610 (M), MED610 with HA coating (MHA), MED610 with PDA coating (MP), and MED610 with PDA/HA coating (MPHA).

biocompatibility of materials is extremely important. The cell proliferation of the hMSCs cultured on M, MP, MHA, and MPHA for 1, 3, and 7 days was evaluated by PrestoBlue assay (Figure 5). The result shows that absorbance of MED610 object with PDA coatings (MP and MPHA) is higher than Ctl and without PDA coatings (M and MHA). Besides, the MED610 object without any coatings shows the lowest absorbance. In addition, the hMSCs cultured on MED610 object with both PDA coatings reveals a higher area of cell adhesion and are flat with an intact, well-defined morphology (Figure 6). These results point that MED610 object with PDA coatings can improve its biocompatibility, making it more suitable as a biomedical material.

3.3 Osteogenic Differentiation

The hMSCs cultured on M, MP, MHA, and MPHA for 3 and 7 days were analyzed the ALP activity to evaluate the

osteogenic differentiation potential which is an important key to determine if the bone formation process is ongoing. Figure 7 shows cells growing on MPHA can express the most ALP levels, and the ALP expression level of MED610 object without coatings was lowest. The results demonstrate that MED610 object with PDA/HA coating helps to improve the osteogenic differentiation potential of stem cells.

4. Discussion

MED610 is a biocompatible 3D printing photocurable material commonly used in medical and dental fields requiring precise visualization and patient contact. Although this material is suitable for over 30 days' skin contact and up to 24 h mucosal membrane or bone contact, it can be more widely used if it can be enhanced

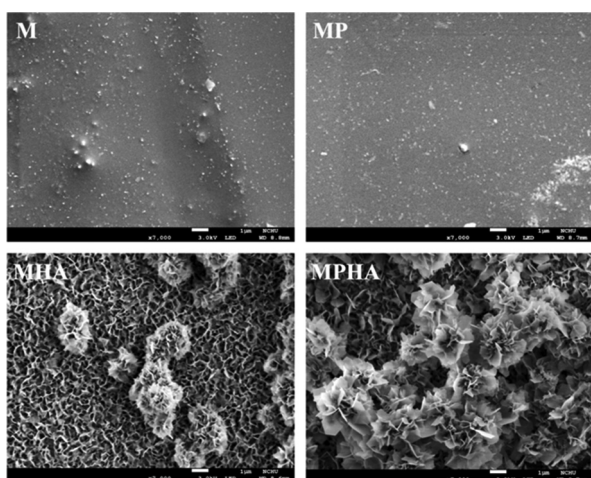


Figure 3. Surface scanning electron microscope images of the MED610 substrate with various coatings.

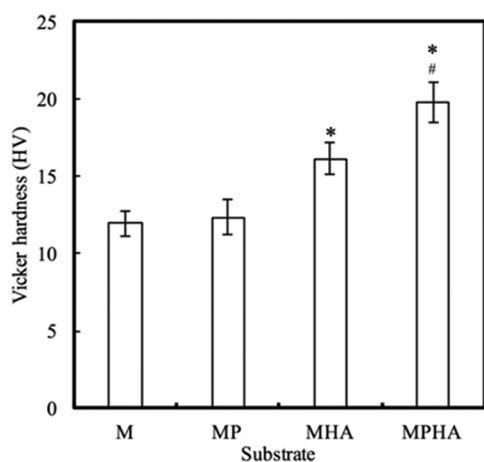


Figure 4. Vickers hardness of the MED610 substrate with various coatings.

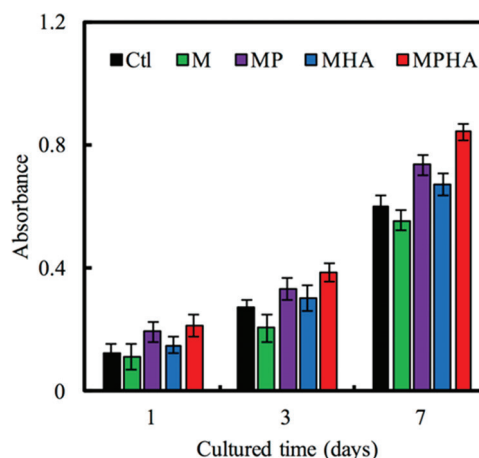


Figure 5. The cell proliferation of human mesenchymal stem cells cultured on the MED610 substrate with various coatings for 1, 3, and 7 days.

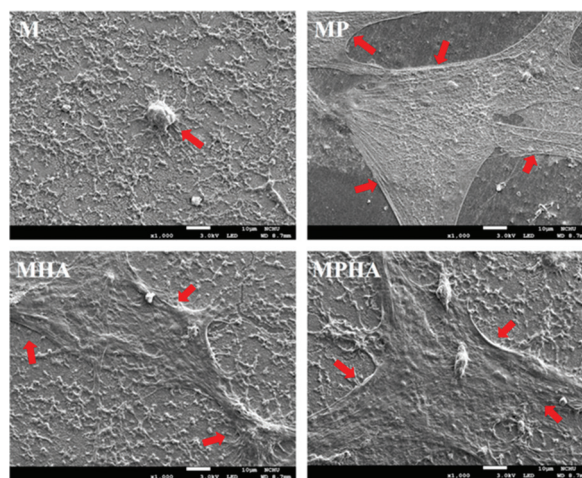


Figure 6. Surface scanning electron microscope images of human mesenchymal stem cells cultured on the MED610 substrate with various coatings. Cells are indicated by red arrows.

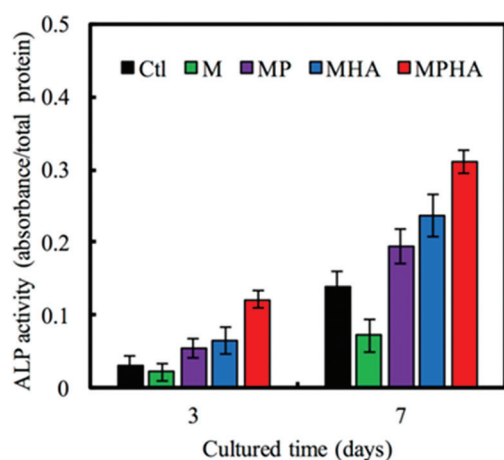


Figure 7. Alkaline phosphatase activity of human mesenchymal stem cells cultured on the MED610 substrate with various coatings for 3 and 7 days.

its biocompatibility and be promoted the potential of cell bone differentiation. Previous studies reported that PDA coatings on biological materials could facilitate cell attachment and promote cell proliferation^[22,23]. When HA was added in PDA coatings, the phenomenon of cell calcium deposition was significantly promoted^[20]. This means that the PDA/HA coatings can help to enhance osteogenic differentiation. In this study, the PDA/HA coatings were applied in MED610 objects. The results show that the biocompatibility, cell morphology, and bone differentiation potential of the cells cultured on the MED610 objects with PDA/HA coating can be improved. This study demonstrates that PDA/HA coating applications also have similar functions^[20] on objects printed with MED610 materials to enhance their applicability.

5. Conclusion

This study shows that using the PDA/HA coating added to the MED610 biomedical 3D printing material that has been used in the medical field can improve its hardness, HA mineralization, cell proliferation, good cell morphology, and the amount of ALP expression. Therefore, the PDA/HA coating enhances the potential and development of MED610 materials for clinical applications in orthopedics and dentistry.

Acknowledgments

The authors acknowledge receipt of grants from the National Science Council grants MOST (107-2218-E-468-005 – and 107-2622-E-468-002-CC2) of Taiwan and China Medical University Hospital grants (CRS-106-021).

Authors' Contributions

Yu-Fang Shen conceived the ideas and organized the main content; Yi-Wen Chen and Hsin-Yuan Fang conducted

the experiments and wrote the manuscript; and Ming-You Shie contributed some detailed techniques.

Conflicts of Interest

The authors declare no competing financial interests.

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