3D bioprinting of a photo-crosslinkable platelet lysate based bioink

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Introduction and Statement of Purpose:

Bioprinting systems are a suitable method to fabricate complex structure with layer-by-layer deposition of cell-laden bioinks, which mimic native organs and tissues. However, Bioinks have several limitations, such as non-degradable, poor physical properties and low stability. To overcome these limitations, we used platelet lysate (PL), which was obtained from whole blood through differential centrifugation, as a biomaterial. As autologous materials, platelets play an important role in hemostasis, bone growth, cartilage repair, and wound healing through the release of growth factors and adhesion molecules during freeze–thaw cycles of platelet-rich plasma (PRP). Despite its tremendous potential, PL presents an inherent fragility and fast dissolution, which hinders its application. To improve these properties, we have developed a tunable bioink, which is made using 3D-printable platelet lysate (PLMA)-based hydrogel that can simulate the 3D structure of tissues and can quickly place the crosslinked hydrogel layer-by-layer to build cell-laden hydrogel constructs through methacrylated photo-polymerization.

Methods, materials and analytical procedures:

Characterization of the PLMA hydrogel properties were examined using rheology, porosity, swelling assay. It was confirmed that the stiffness of PLMA hydrogel can be adjusted according to the degree of photo-crosslinking. Cell viability and proliferation demonstrated that the PLMA-based scaffold is biocompatible when applied as a bioink. To form multilayer cell-laden scaffolds, cells were trypsinized, counted, and placed in Eppendorf tubes (GFP-hMSCs and RFP-HUVECs [5×10^6 cells ml^{-1}]). Further, we developed multilayer cell-laden scaffolds with the PCL–PLMA through photo-crosslinking to fabricate composite tissue. To improve printability of 3D complex shapes, PLMA was blended with different additive materials. To confirm the effect of UV treatment after degradation, the gelatin-PLMA blend was soaked in DW at 50 ◦C, while the alginate blending PLMA was tested using sodium citrate solution.

Results:

We synthesized PLMA through methacrylation to enhance mechanical property and prepared hydrogels via photo-crosslinking. We determined that the relationship between porosity, mechanical properties, rheological measurements were performed for various PLMA concentrations and UV irradiation times (Fig 1). To fabricate composite tissues, we used PCL as a frame for the printing of PLMA, and also designed artificial blood vessel channels to engineer vascularized complex tissues. As a result, we observed the internal microporous structure between the frames of the multilayer printed scaffolds, which enables penetration of water and nutrients for cell growth. and we confirmed the presence of encapsulated cells in PLMA bioink (Fig 2). We printed cells into multi-layers which were organized by MSC, MH pattern. The cells proliferated with no indications of damage for up to 14 days and presented a stretched morphology indicating good interactions with each other (Fig 3). We developed a frame-free system to form multi-layered structures and various shapes. To increase viscosity, PLMA was blended with different additive materials. With UV treatment for 90 s, 3D printed gelatin or alginate/PLMA structure were well preserved in its original state without degradation.

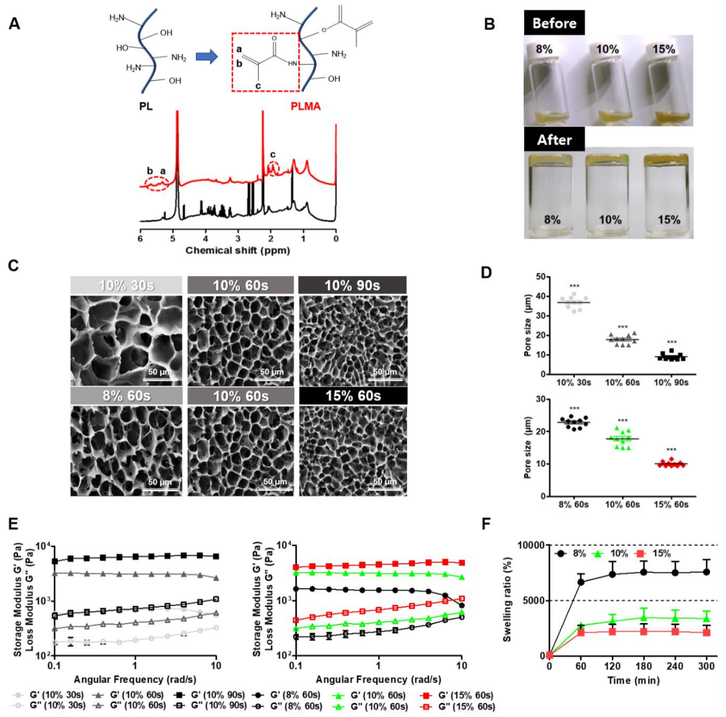

Figure 1. Characterization of PLMA hydrogels. (A) ^{1}H NMR spectra of PLMA as compared to a pristine PL, (B) optical images of cross-linked PLMA hydrogels (top : non-crosslinked, bottom : crosslinked), (C) SEM images of PLMA hydrogels with different concentration and UV irradiation time (magnification x 800), (D) quantification of porosity from all PLMA hydrogel groups by image analysis (n = 10), (E) the storage modulus (G') and loss modulus (G") on the frequency of PLMA hydrogels with various UV irradiation time and concentration, and (F) swelling analysis of PLMA hydrogels with various concentration.

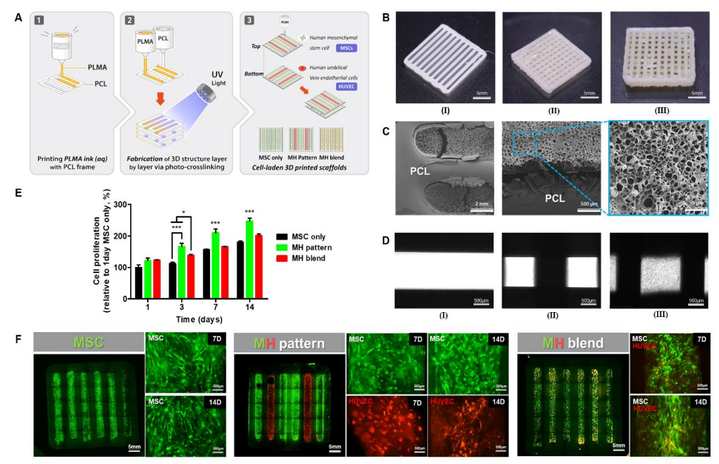

Figure 2. 3D printed microstructre composed of PLMA hydrogel and PCL frame: (A) Schematic illustration of printing process of multilayer cell-laden scaffolds with PLMA/PCL, (B) optical images of 3D printed PLMA bioink with PCL frame (Ⅰ), multilayer printed scaffolds (Ⅱ), multilayer cell-laden scaffolds (Ⅲ), (C) SEM images of PLMA hydrogels with PCL scaffolds, (D) microscopic images of 3D printed PLMA ink with PCL frame (Ⅰ), multilayer printed scaffolds (Ⅱ), multilayer cell-laden scaffolds (Ⅲ), (E) proliferation rate of hMSCs encapsulated in PLMA hydrogel by CCK-8; * indicates a significant difference at $p < 0.05$, *** indicates a significant difference at $p < 0.001$, (F) representative fluorescence images of 3D bio-printed scaffolds with encapsulated with GFP-MSC and RFP-HUVEC.

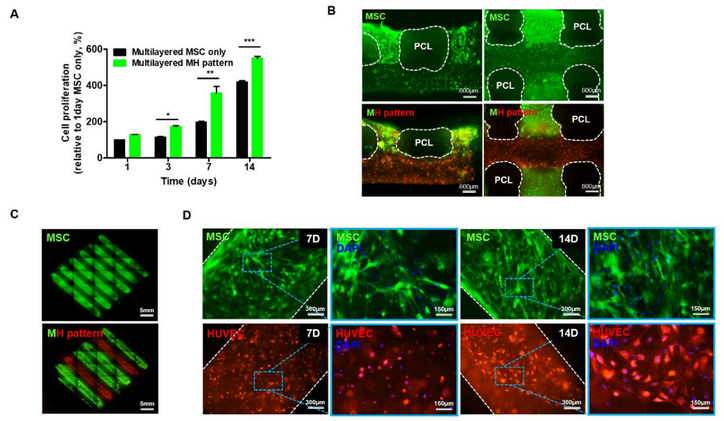

Figure 3. *In vitro* biocompatibility of 3D bio-printed constructs with various cell types. (A) proliferation rate of various cells encapsulated in PLMA hydrogel, (B) representative fluorescence images of 3D multi-layered bio-printed scaffolds encapsulated various cell types and, (C) Overall stack image on 14th days (Top-view), (D) magnification images of cell proliferation in MH pattern for a long period of time.

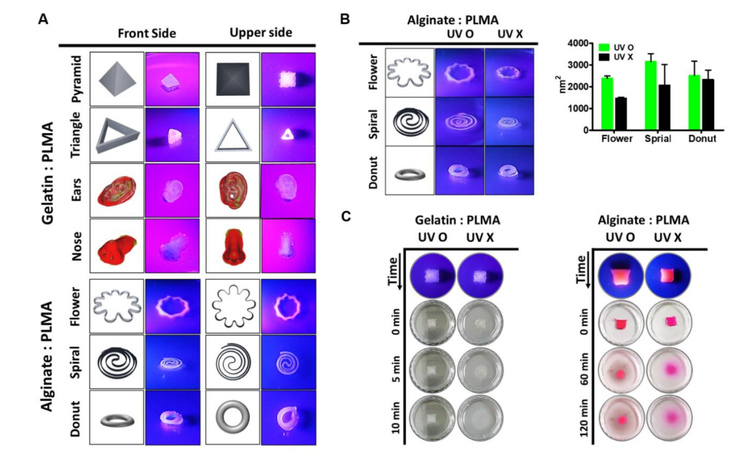

Figure 4. 3D printing of multilayer frame-free scaffold with viscous biomaterials. (A) representative optical images of 3D bio-printing with PLMA/Gelatin and PLMA/Alginate ink, (B) the comparison of with UV treatment effect at PLMA/Alginate ink, (C) the degradation of the effect of UV treatment after degradation at Gelatin/Alginate, PLMA/Alginate ink over time.

Conclusions:

In this study, we confirmed that the multi-layer scaffolds fabricated by bio-printing not only have a stable structure but also a porous 3D construct in which encapsulated cells in PLMA ink can growth. Finally, we suggest that the developed PLMA bioink is considered to be a novel bioink with numerous potentials and can be applied in the field of tissue engineering. Finally, we pre-sent photo-polymerizable hydrogels, which are based on PLs derived from blood, as novel bioinks in tissue engineering.

Acknowledgements:

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