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Patient-specific bioinks for 3D bioprinting of tissue engineering scaffolds

Negar Faramarzi, Iman K. Yazdi, Mahboubeh Nabavinia, Andrea Gemma, Adele Fanelli, Andrea Caizzone, Leon M. Ptaszek, Indranil Sinha, Ali Khademhosseini, Jeremy N. Ruskin*, Ali Tamayol*

[*] Corresponding authors: A. Tamayol and J. Ruskin

Dr. N. Faramarzi, Dr. I. K. Yazdi, Ms. M. Nabavinia, Mr. A. Gemma, Ms. A. Fanelli, Mr. A. Caizzone, Prof. A. Khademhosseini, and Prof. A. Tamayol

Biomaterials Innovation Research Center

Department of Medicine

Brigham and Women's Hospital

Harvard Medical School, Boston, MA 02139, USA.

Dr. N. Faramarzi, Dr. I. K. Yazdi, Ms. M. Nabavinia, Mr. A. Gemma, Ms. A. Fanelli, Mr. A. Caizzone, Prof. A. Khademhosseini, and Prof. A. Tamayol

Harvard-MIT Division of Health Sciences and Technology

Massachusetts Institute of Technology

Cambridge, MA 02139, USA.

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Dr. N. Faramarzi, Dr. L.M. Ptaszek and Prof. J.N. Ruskin

Department of Medicine

Massachusetts General Hospital

Harvard Medical School, Boston, MA, 02114, USA

E-mail: jruskin@mgh.harvard.edu (J. Ruskin)

Dr. I. K. Yazdi, Prof. A. Khademhosseini and Prof. A. Tamayol

Wyss Institute for Biologically Inspired Engineering

Harvard University, Boston, MA 02115, USA.

Dr. I. Sinha

Department of Surgery

Brigham and Women's Hospital

Harvard Medical School, Boston, MA, 02115, USA.

Prof. A. Khademhosseini

Center of Nanotechnology

King Abdulaziz University

Jeddah 21569, Saudi Arabia

Prof. A. Khademhosseini

Department of Bioengineering

Department of Chemical and Biomolecular Engineering

Department of Radiology

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California NanoSystems Institute (CNSI)

University of California

Los Angeles, CA 90095

Prof. A. Tamayol

Department of Mechanical and Materials Engineering

University of Nebraska, Lincoln

Lincoln, NE, 68588, USA

E-mail: atamayol@unl.edu (A. Tamayol)

ABSTRACT: Bioprinting has emerged as a promising tool in tissue engineering and regenerative medicine. Various three dimensional (3D) printing strategies have been developed to enable bioprinting of various biopolymers and hydrogels. However, the incorporation of biological factors has not been well explored. As the importance of personalized medicine is becoming more clear, the need for the development of bioinks containing autologous/patient-specific biological factors for tissue engineering applications becomes more evident. We used platelet-rich plasma (PRP) as a patient-specific rich source of autologous growth factors that can be easily incorporated to hydrogels and printed into 3D constructs. PRP contains a cocktail of growth factors enhancing angiogenesis, stem cell recruitment and tissue regeneration. Here, we report the development of an alginate-based bioink that can be printed and crosslinked upon implantation through exposure to native calcium ions. This platform can be used for the controlled release of PRP-associated growth factors which may ultimately enhance vascularization and stem cell migration.

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Keywords: Platelet rich plasma (PRP); Alginate-based hydrogels; Patient-specific bioinks; Tissue engineering; 3D bioprinting.

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Introduction

Three-dimensional (3D) printers have been a major source of advancements in many areas of engineering and technology development. The ability of 3D printing to create acellular and cell-laden scaffolds with pre-designed patterns, architecture and distribution of cells and biological factors has fueled important research directed at solving challenges in the field of tissue engineering and regenerative medicine [1]. As a result, considerable attention has been focused on developing strategies to facilitate 3D printing of a variety of hydrogels and biopolymers with suitable resolution [2, 3]. In addition, a significant body of research has focused on developing biologically relevant bioinks [3]. Bioink is typically referred to biomaterials that carry cells and are being printed into 3D scaffolds or tissue like structures; bioinks are a crucial component of any bioprinting effort [3, 4]. Among various biopolymers, hydrogels have been widely used in developing tissue engineering scaffolds due to their similarity with native extracellular matrix (ECM) and their tunable physical properties and degradation profile [5]. Alginate is among the most popular hydrogels used in fiber-based technologies, which is due to its rapid and reversible crosslinking in presence of calcium ions into hydrogels with strong mechanical properties [6]. Alginate is also FDA-approved for many biomedical applications and has been used in a number of clinical trials [7].

Several methods have been proposed to further improve the biological function of alginate hydrogels including the incorporation of electrically conductive nanomaterials to improve cell-cell interactions as well as its functionalization by RGD peptides to improve cell-substrate interactions [8]. Tamayol et al. recently reported on the development of a robust approach for fabricating fibers and constructs from various hydrogels in which alginate was used to create a sacrificial template [9, 10]. The entrapped pre-polymer solution was then crosslinked and alginate could be removed from the construct if needed. They also proposed

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an alternative approach for printing of protein-based hydrogel fibers containing a sacrificial polymeric network (alginate), in which $CaCl_2$ mist was used to form the fiber templates during the printing process and a secondary polymerization step was used to create hydrogel constructs [10]. This approach has been adopted by a number of other research groups, which further demonstrates the importance of alginate-based bioinks [11].

The administration of growth factors has shown a great promise for partial repair of damaged tissue and restoration of normal physiological function [12]. Growth factors can be administered locally or systemically with the aim of stimulating angiogenesis, stem cell recruitment and differentiation, cell survival and proliferation, reduction of apoptosis and adaptive remodeling [12, 13]. Prefabricated scaffolds create a unique opportunity for localized delivery of growth factors at the site of tissue injury [14]. There are a number of studies reporting on the 3D printing of factor-eluting scaffolds [15]. However, a key limitation is that these scaffolds usually release a single factor and, depending on their source, these proteins are expensive and can result in disease transmission or inflammation. Thus, bioinks that are based on immunologically compatible materials such as alginate and patient-specific growth factors could address this key challenge. In addition, preserving the stability of the incorporated factor during the fabrication and implantation of the scaffold is not feasible. Platelet rich plasma (PRP) is a plasma fraction containing several growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), tumor growth factor (TGF), insulin-like growth factor (IGF), and stromal cell-derived factor (SDF), all of which play important roles in inducing angiogenesis and the recruitment of stem cells [16, 17]. PRP has been shown to release biologically active proteins and growth factors over several days and has been widely used as a treatment for musculoskeletal disorders [18]. There are also a small number of animal studies using PRP in the treatment of cardiovascular disease [19, 20]. Thus, a bioink based on alginate and PRP could potentially be used for 3D bioprinting of scaffolds for application in musculoskeletal and cardiovascular tissue engineering.

In this study, we developed an alginate-based bioink incorporated with PRP as a source of

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growth factors for printing 3D scaffolds and tissue engineering constructs. The concentration of PRP was optimized and the effectiveness of the released factors was evaluated. This novel bioink can be used for engineering scaffolds that can induce a healing response in cardiovascular and musculoskeletal tissue constructs.

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Results and Discussions

The goal of this study was to develop a bioink that is capable of eluting patient-specific growth factors. PRP contains high concentration of platelets and its use mimics the human body's response to injury, in which activated platelets release a number of growth factors and cytokines essential for inducing the healing process. Local PRP delivery has shown favorable outcome in the treatment of some musculoskeletal disorders [21]. In order to engineer this bioink with patient-specific growth factors, PRP was extracted from blood sources and then mixed with alginate (**Figure 1**a). Alginate is a hydrogel widely used in 3D bioprinting of complex constructs. In addition, alginate-based inks also can be combined with other proteins or polymers to fabricate interpenetrating polymeric network (IPN) materials. Sodium alginate forms a viscous solution and as soon as it is in contact with Ca^{2+} ions, solid hydrogels of Ca-alginate, typically referred to as alginate, is formed. One approach for enabling the 3D printing of alginate-based bioinks is through printing in solutions or nanodroplets containing $CaCl_2$. The latter approach does not require modification of commercially available 3D printer nozzles and can result in higher resolution. To form stable constructs using alginate-based bioinks and achieve acceptable resolution, a low concentration of $CaCl_2$ can be mixed with the bioink to form partially crosslinked alginate which is highly viscous yet injectable through the printer head. The ink can be extruded through the nozzle and, as the fibers are being deposited on the substrate in an environment filled with $CaCl_2$ fume, the other layer of the fibers will be crosslinked to form stable fibers (Figure 1b). Immediately after printing, the fibers can be fully crosslinked using $CaCl_2$ solution. By connecting the printer to different reservoirs containing two different bioinks, constructs with controlled patterns of different

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inks can be fabricated (Figure 1c).

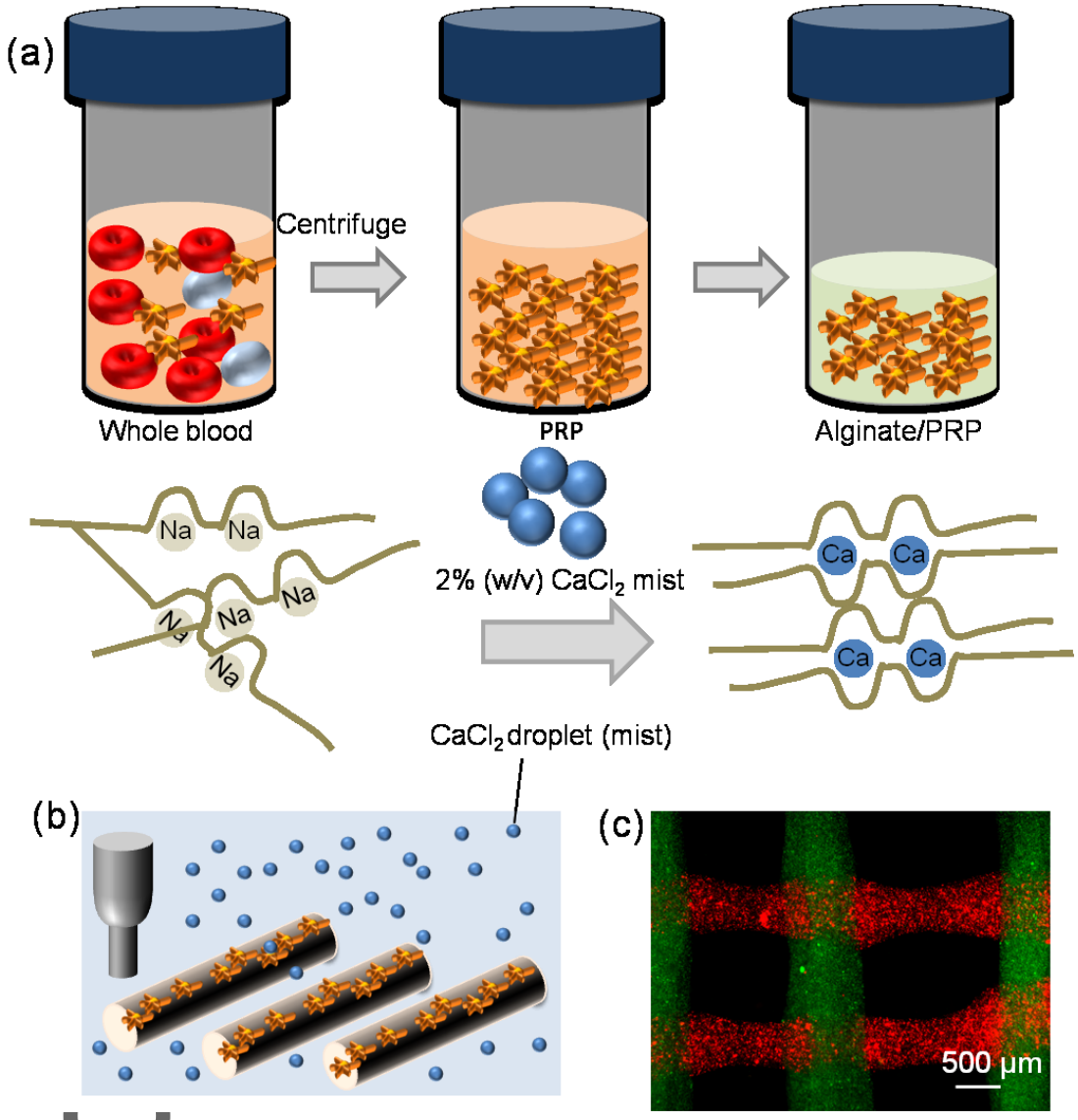

Figure 1. Schematic of the proposed patient-specific bioink. (a) Schematic of the extraction of PRP and its incorporation in alginate to form the bioink. (b) Schematic of the proposed bioprinting process. (c) A typical bioprinted scaffold from alginate-PRP containing fluorescent particles.

The physical properties of the engineered ink and the crosslinked constructs are important in determining printability of the ink and also the suitability of the crosslinked hydrogels for long-term culture of cells. Previous studies suggested that at high concentrations PRP could

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induce inhibitory effects on cell growth and tissue regeneration both in vitro and in vivo [22]. In the present study, the concentration of PRP in the alginate-based bioink was maintained at 50 U/mL of bioink, which is within the range recommended in the literature [19, 23]. We assessed the mechanical properties of fully crosslinked PRP/Alginate hydrogels. Circular disks (6 mm in diameter and 2 mm in height) were fabricated as described in the Experimental Section. Briefly, the 100 µL of bio-ink was added to PDMS molds and then was covered by a flat agarose sheet containing 2% (w/v) $CaCl_2$. The bioink was slowly crosslinked over 10 min into disks with flat surfaces. A compressive mechanical test was conducted and the compressive modulus of samples with and without PRP (50 U/mL of bioink) was determined (**Figure 2**a). The results demonstrated that PRP slightly increased the compressive modulus of the crosslinked bioinks to ~110 kPa. This increase might be due to the formation of crosslinked network of activated platelets. Other important parameters that were assessed included the degradation and water uptake capacity of the lyophilized crosslinked bioinks containing different concentrations of PRP. The presence of PRP enhanced the water-uptake of the bioink; thus, it is expected that construct containing PRP may swell slightly more than pristine hydrogels (Figure 2b). The degradation rate of the bioink containing PRP was slightly faster than pristine hydrogel (**Figure S1**). The addition of PRP to alginate slightly reduced the viscosity of the formed bioink, which was not statistically significant (**Figure S2**). Rheological measurements revealed that the addition of PRP to alginate solution did not affect the G′ and G" values significantly. While our data demonstrated that PRP addition has an effect on the bioink gelation, which can be used to modulate its mechanical stiffness. The data was in agreement with the observation in the compressive modulus of the bioinks.

Upon activation of platelets in PRP, a cocktail of biological growth factors is released which enhances tissue healing [17]. The goal of this research was to engineer a bioink that could control the release of these factors by incorporation of patient-specific PRP. Thus, we assessed the total rate of protein release from the engineered bioinks. Since alginate does not contain proteins, all the released proteins from the constructs were directly eluted by the

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encapsulated platelets. We compared the rate of protein release from alginate-based bioink containing PRP with PRP that was gelled by the addition of $CaCl_2$ (Figure 2c). To determine the release, 100 μL of the crosslinked bioink or PRP was placed in an 8 um transwell at the interface of 1 mL of solution in 12-well plates. The results demonstrated that the release rate of total protein was slightly (not statistically relevant though) slower from the bioink compared to the PRP gel. In addition, the total protein release from the bioink was similar to that seen from the PRP gel. Similar to many hydrogel systems, the engineered bioink had an initial burst release followed by a gradual release of proteins over 120 hr. We also assessed the activity of the released factors. In particular, we measured the release rate of active VEGF (42 kDa), which is one of the key factors in angiogenesis (Figure 2d). The results showed that VEGF release followed a profile similar to that of other proteins and a release rate of approximately 1000 pg/mL was achieved, when released in 2 mL solution. The localized release of this protein may ultimately enhance the rate of angiogenesis in injured tissues.

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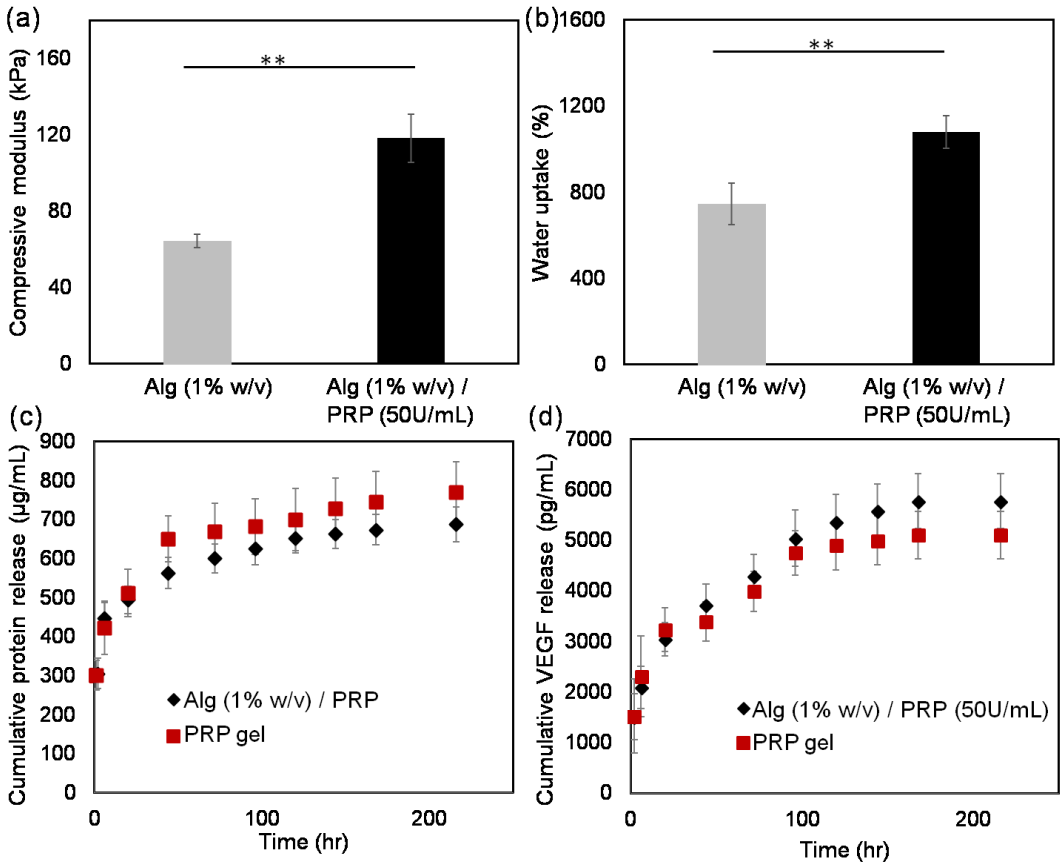

Figure 2. Physical characterizations of alginate/PRP hydrogels and assessment of protein release profile. (a) Compressive modulus of alginate (1% w/v) and alginate (1% w/v)/PRP (50U/mL) hydrogels. (b) Water uptake of Alginate and Alginate/PRP hydrogels in PBS-based solution after 24 hours. (c) Cumulative total protein release from alginate/PRP (50U/mL) hydrogel and PRP gel. (d) Cumulative VEGF release from alginate/PRP hydrogel (50U/mL). (**: P<0.01).

One of the key biological effects of platelets after tissue injury is the release of factors that are essential for recruitment of immune cells as well as stem cells to the injury site to initiate the healing cascade. Similarly, PRP releases a range of factors including SDF-1 that affect various stem cells and induce their migration. In addition, PRP releases a cocktail of factors that also assist in the maintenance of recruited cells. Accordingly, we evaluated the effect of PRP concentration on the metabolic activity of mesenchymal stem cells (MSCs). Different volumes of bioink composed of 1% (w/v) alginate and 50 U/mL of PRP was crosslinked and

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added to cultures of MSCs in basal medium. While the same bioink (i.e. carrying PRP with concentration of 50 U/mL of bioink) was utilized, the addition of the culture medium reduced the concentration of PRP within the culture environment. The final concentration of the PRP in the culture environment depended on the volume ratio between the bioink and the culture medium. The maximum concentration of PRP was achieved by utilizing the same volume of bioink and culture media, which resulted in the 25 U/mL of culture media. The cultures were then incubated for 5 days and the metabolic activity of the cultures was measured using the PrestoBlue assay (**Figure 3**a). The results demonstrated that PRP had a positive effect on cellular growth. In addition, no inhibitory effect on cell growth was observed up to a concentration of 25 U/mL of PRP in the MSC culture medium. We also assessed the effect of PRP on cell recruitment and cellular migration by two different assays: 1) scratch assay [24] and 2) Boyden chamber migration assay [25]. For the scratch assay, a monolayer of MSCs was created and an approximately 200 µm wide scratch was created in the culture. Crosslinked bioink was placed in the wells with in a volume resulting in a total concentration of 25 U/mL in the culture medium. The rate of scratch closure was compared by microscopy after 10 and 24 hr (Figure 3b, c). The results showed that the presence of PRP significantly enhanced the rate of scratch closure in comparison to alginate bioink without PRP as the negative control. This is aligned with previous observations reported in the literature demonstrating the important role of PRP-induced growth factors including SDF-1 on mesenchymal cells proliferation and migration rate[26]. The migration assay was also assessed through a Boydon chamber assay. Cells were seeded inside a transwell culture insert with pore size of 5 µm. Crosslinked bioink containing 50 U/mL of PRP was fabricated and placed in the bottom of the chamber such that a total concentration of 10 and 25 U/mL of PRP in the culture media was achieved (Figure 3d). The results confirmed a positive role of PRP on cellular migration. In addition, within the tested concentration range of PRP, no inhibitory effect was observed (Figure 3d).

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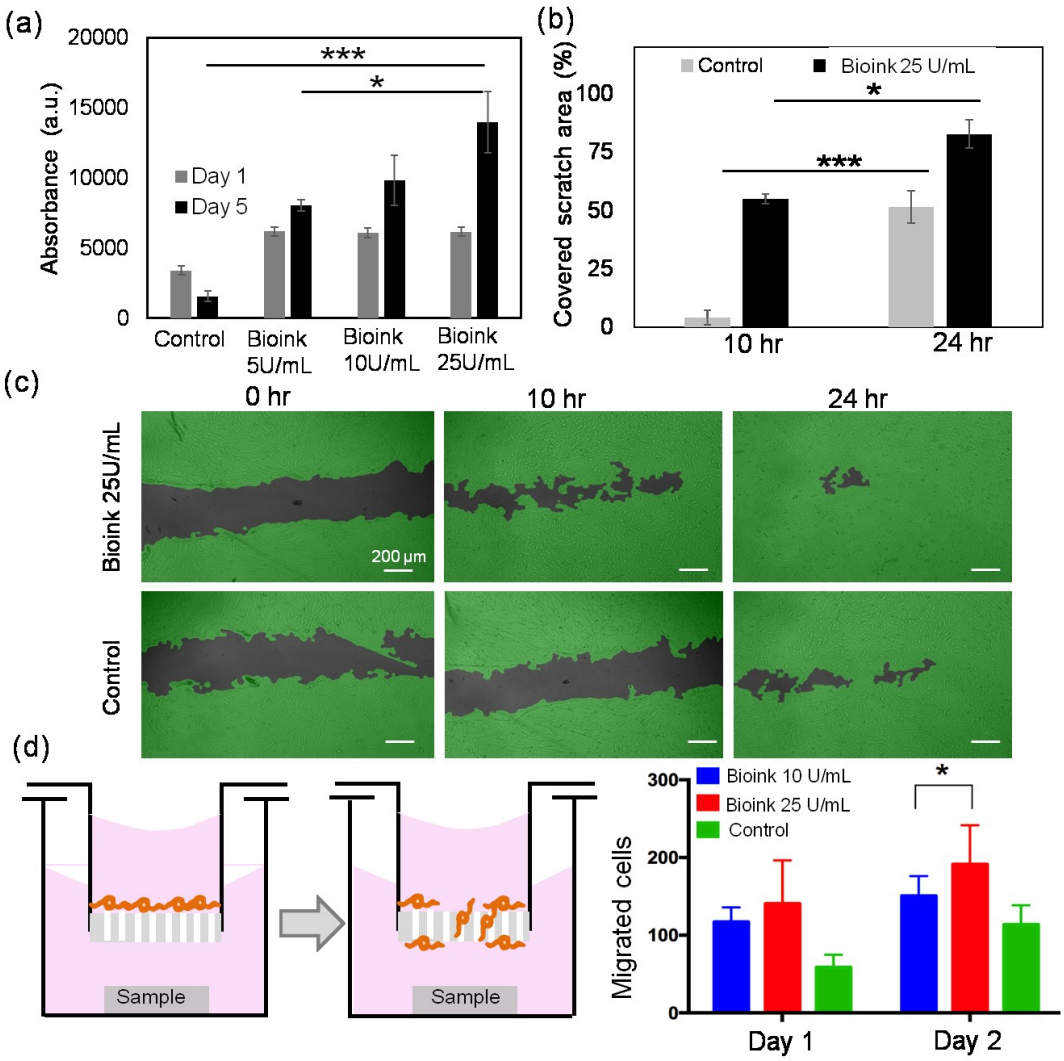

Figure 3. Effect of PRP-based bioink on the growth and function of MSCs. (a) Metabolic activity of MSC cells treated with alginate and alginate/PRP. (b, c) Scratch assay demonstrating the effect of PRP on migration and reorganization of MSCs in culture in comparison to the alginate as the negative control. (d) Schematic of migration assay using Boyden chamber and migration of MSC cells in the presence of chemo attractants released from alginate/PRP hydrogel. (*: P<0.05; **: P<0.01; ***: P<0.001)

Another important biological process that is essential for tissue healing and regeneration is angiogenesis. Platelets in physiological conditions after an injury initiate this process through the secretion of angiogenic factors such as VEGF, PDGF, and TNF-a [27]. We assessed the effect of PRP and the released proteins on the activity of human umbilical vein endothelial

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cells (HUVECs). The PrestoBlue data reflecting metabolic activity demonstrated a significant boost in the growth of HUVECs in the presence of PRP (**Figure 4**a). Based on the release data presented in Figure 2 a predicted VEGF concentration of approximately 50 ng/mL would be achieved, which is sufficient to significantly improve the growth of HUVECs. VEGF and other angiogenic factors can also play a role on the function of HUVECs. Such effect was assessed using a standard tube formation assay (Figure 4b)[28]. The results showed that the factors released from alginate/PRP-based bioinks significantly improved the length and complexity of the formed tubes (Figure 4c, d).

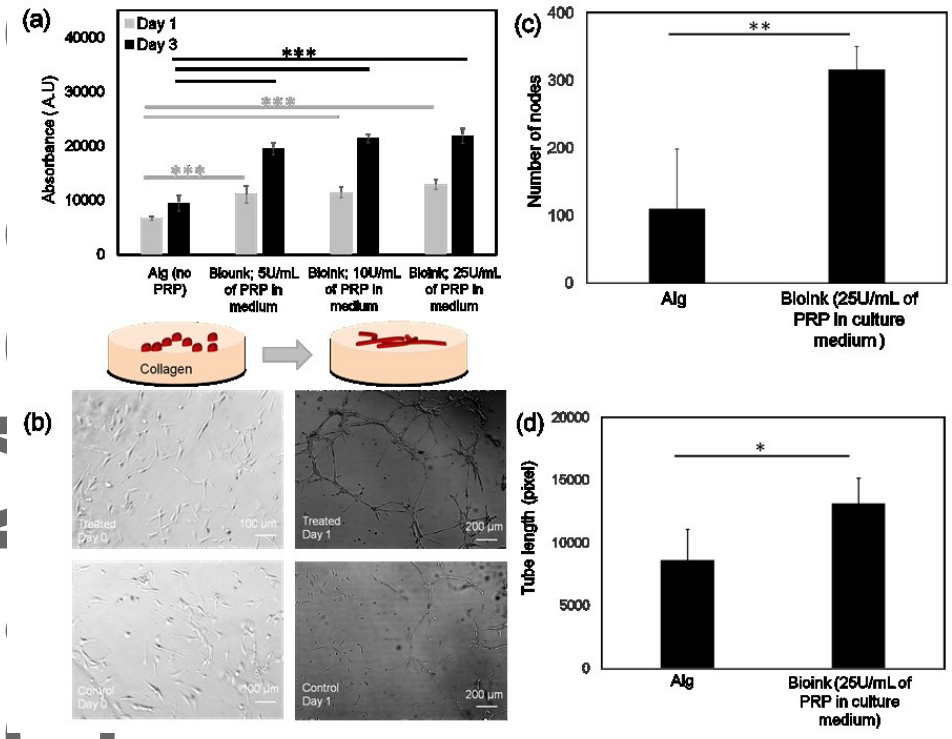

Figure 4. (a) Metabolic activity of HUVECs treated with alginate and alginate/PRP over 3 days of culture in media without any growth factors; alginate is the negative control. (b) A typical tube formation assay for assessment of the function and morphology of HUVECs. HUVECs were cultured on collagen type I and treatment (alginate/PRP) was applied and the number of branch points and tube length were measured. (c, d) Summary of the total number of branch points measured in five fields under each condition. The micrographs confirmed a significant difference in cellular morphology. (*: $P<0.05$; **: $P<0.01$, ***: $P<0.001$)

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We assessed the printability of the developed bioink using a commercial 3D printer (BioBots, MA). The bioink with a composition of 1% (w/v) alginate, 50 U/mL PRP, and 0.04% (w/v) $CaCl_2$ was prepared and loaded into the reservoir of the printer. To improve the quality of printings, instead of extruding into a $CaCl_2$ bath, a humidifier was used to generate $CaCl_2$ fume formed from nanosized droplets. The fume achieved rapid partial-crosslinking of the printed bioink. The fabricated constructs were then immersed into 2% (w/v) $CaCl_2$ solution. Three different designs including: 1) grid structure, 2) tree-like structure similar to tissue vasculature, and 3) serpentine lines were printed (**Figure 5**). The nominal dimensions and the fabricated constructs are shown in Figure 5a,b. It can be seen that the difference between the intended design and the fabricated construct is approximately ±200 um, which is comparable to the resolution of the 3D printer (±100 um). The electronic design and the fabricated constructs for the tree-like and serpentine structures are shown in Figures 5c,d and 5e,f, respectively. The difference between the intended design and the fabricated constructs was less than ±100 um.

We also assessed the possibility of engineering stable free standing 3D printed constructs. After printing, the constructs were peeled off using a blade without losing their physical integrity (Figure 5g). The constructs were maintained in aqueous solutions for 24 hr at 37°C and it was observed that their geometrical features were preserved during the incubation period (Figure 5h,i). Overall, the results suggest that the engineered bioink can be printed into 3D constructs that are easy-to-handle. The possibility of mixing patient-specific cells with the developed bioink enables engineering constructs in which all the biological components are patient specific to minimize the chance of significant adverse immune response after their implantation.

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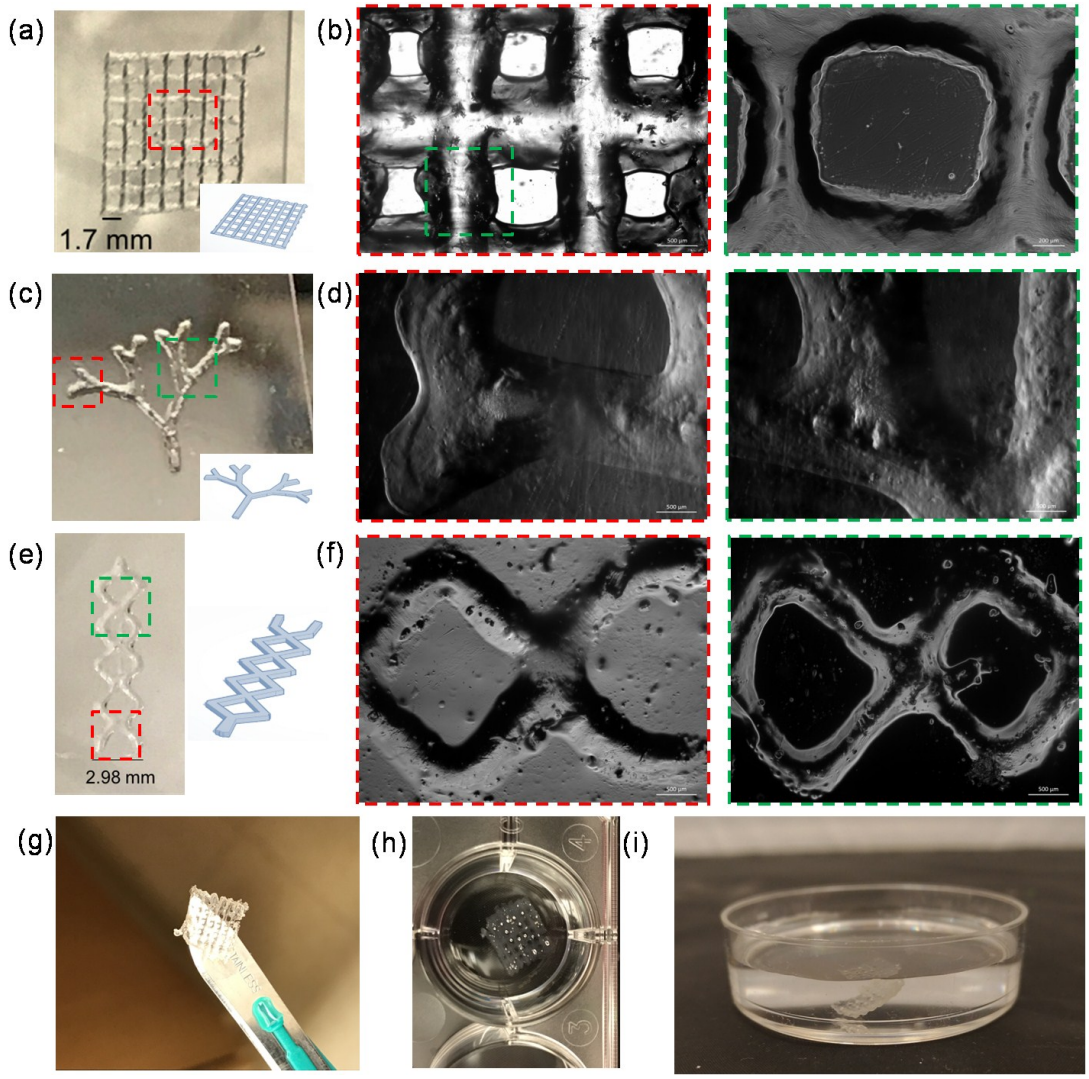

Figure 5. 3D bioprinting of the engineered bioink. (a-f) Images and micrographs of different constructs based on alginate and PRP. In the fabrication of these constructs a solution of 1% (w/v) alginate, 50 U/mL of PRP, and 0.04 % (w/v) $CaCl_2$ was used. The digital files are also shown in (a,c,e). (g-i) 3D constructs could be detached from the substrate without losing their integrity and could be used as free standing constructs.

Conclusions

Despite recent advances in the field of bioprinting and bioinks, the incorporation of growth

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factors in these inks in a way that it does not induce an immune response has not been demonstrated. PRP has been widely investigated as a biological source of growth factors that can be harvested from individual patients to minimize the host immune response. PRP releases a cocktail of factors that induce a range of physiological processes that are essential for tissue healing. In this study, PRP was incorporated into alginate which is a biocompatible FDA-approved hydrogel frequently used in bioprinters. The incorporation of PRP slightly increased the compressive modulus of the bioink. The bioink had a gradual release of various proteins and growth factors over several days. In vitro experiments demonstrated that the bioink containing PRP can positively affect the function of two important populations of cells (MSCs and ECs), which are involved in tissue healing processes. The printability of the engineered bioink was demonstrated by fabrication of various constructs. This bioink can be readily utilized by any extrusion-based 3D printer. The developed bioink and the fabricated constructs based on this formulation may prove to be useful in the treatment of injured tissues *in vivo*. In addition, bioinks containing PRP can facilitate autologous and personalized therapies.

Experimental Section

Materials

All chemical and cell culture media and reagents were purchased from Sigma-Aldrich and Invitrogen, respectively unless mentioned otherwise.

PRP preparation

Human blood was centrifuged at 250 gravity for 15 min to separate red blood cells from plasma. The upper plasma phase, including the interface, was centrifuged at 1600 gravity for 10 min to pellet the platelets. The platelets were suspended in the platelet-poor plasma supernatant to create platelet-rich plasma with the concentration of $\sim 1\times10^6$ platelet/μL. Platelet counting was done with Sysmex® XN-10 automated hematology analyzer.

Formation of the bioink

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Solutions of semi crosslinked alginate/$CaCl_2$ (1%/0.025% (w/v)) were prepared. The solution was sterilized under UV overnight for biologic experiments. PRP was mixed thoroughly with sodium alginate solution for making Alginate/PRP with concentration of 50 U of PRP per mL of the bioink. For physical characterization, the bioinks were used to form cylindrical blocks, 6 mm in diameter. $CaCl_2$ (2% w/v) was pre-filtered through a sterile 0.2 mm membrane and then 3% (w/v) of agarose was dissolved in that. The solution was solidified in PDMS molds to form sheets at 4 ℃. The bioink solutions were then covered with the agarose gel containing $CaCl_2$ for 60 minutes. Each disc was rinsed gently with PBS gently.

Mechanical characterization of the bioink fabricated constructs

Hydrogel disks with different concentrations of PRP were fabricated as described above and their compressive mechanical properties were measured at room temperature using an Instron 5542 mechanical tester (Norwood, MA, USA) with a 1 kN load cell following the procedures reported in the literature [29]. Samples were placed between two flat grips and were compressed at a strain rate of 1 mm/min. The compressive modulus was determined as the slope of the linear region of the stress-strain curve corresponding with 0-5% strain.

Rheological characterization of bioink

The rheological characteristics of the gel with time (storage, loss modulus and complex viscosity) were measured using a AR-G2 rheometer (TA Instruments). A 20 mm diameter parallel plate geometry with a gap height of 200 μm was used for time sweeps and mineral oil was placed around the circumference of the plate to prevent evaporation. Gels were prepared as described above and deposited directly onto the base and the plate was subsequently lowered. The experiment was performed using a time sweep test at 37 ℃ with a 5% strain and 10 rad/s angular frequency. All measurements were timed to 5 min.

Assessment of the degradation and water uptake of the hydrogels

Alginate/ PRP discs were lyophilized for 48 hr and their dry weight (W_d) was measured (n= 6 for each group). Dry discs were incubated in PBS at 37° C for 24 hr. PBS buffer was removed completely and the hydrogel discs were dried using a napkin and then weighed to determine their water uptake after 24 hr (W_i). Water uptake was calculated using the following formula ((W_i-W_d)/W_d ×100). For degradation experiments, bioink disks were

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placed in PBS and their wet weight was measured over 5 days. The numbers were used to calculate the mass loss with respect to their initial wet Wight.

3D printing of constructs using the engineered bioink

3D constructs were fabricated by sequential fiber deposition using a BioBots Beta (BioBots, Philadelphia, Pennsylvania) (bio)printer. This printer uses a pneumatic system manually controlled. The extrusion head moves both on the XY plane and along the Z-axis over a static platform. It consists of a syringe (both 10ml and 5ml are compatible with this printer) inserted in a support connected to an air compressor and controlled by the software (Repetier Host v1.6.2). Constructs were designed with open source CAD software, Tinkercad (Autodesk, Inc., 2016). The printing process was performed at room temperature. The pressure of the extrusion system was maintained below 1 bar. The constructs were printed either on poly(L-lysine) (PLL) coated glasses to avoid the detachment of the constructs during the printing process. Briefly, glass slides were cleaned with NaOH (1:9 dilution in de-ionized water) for 30 minutes, followed by ethanol and de-ionized water rinsing. The slides were then exposed to oxygen plasma for 2 minutes on both sides. The reactive surface was then immersed in 0.1% (w/v) solution of PLL in deionized water for 10 min and left to dry overnight.

Assessment of release profile of active compounds from Alginate/PRP hydrogels

The total protein release from the engineered alginate/PRP bioink was evaluated. A sample disc of Alginate/PRP was immersed in 1000 mL of PBS at 37°C. For each group, the assay samples were taken in (n = 6). At each time point, 200 µL of the solution was removed and replaced with same amount of PBS for period of analysis. The protein concentration in the collected supernatant was analyzed by the Micro-BCA™ assay. The results are presented as cumulative release as a function of time. VEGF release was assessed by Human VEGF 165 Standard TMB ELISA Development Kit (Peproteck, USA).

Cell culture

HUVECs and human MSCs were cultured and used up to passage 6. MSCs were cultured in α-MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamin, 0.2 mM ascorbic

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acid,100 U/ml penicillin-streptomycin, and 1 ng/ml bFGF. HUVECs were cultured in endothelial culture media obtained from Lonza.

10^4 Human MSC (passage 4) and 1.5×10^4 HUVEC cells passage number less than 6 were cultivated separately on collagen coated 24 well plates (Corning In corporation, Corning, NY). For MSC cells, each well plate contained 500 µl media containing α-MEM, 10% fetal bovine serum, 2 mM L-glutamin, 0.2 mM ascorbic acid, 100 U/ml penicillin and 100 mg/ml streptomycin, Basic medium plus 1 ng/mL bFGF. HUVEC media without VEGF was added for HUVEC cells. Transwell was placed in each well and alginate/PRP disc was placed on the top of the membrane. Presto reading was done according to the protocol provided by company.

To perform the scratch assay, 10^5 MSC were seeded on each collagen coated well plate (48 well plates). When cells reached monolayer confluency, an approximately 200 µm wide scratch was made with a P200 pipet tip in the cell layer. Alginate/PRP or alginate discs were placed on the top membrane of the transwell and MSC media was added to each well. Imaging was done at 10 hr and 24 hr after treatment.

The effectiveness of the released proteins in inducing vascularization was assessed using a standard tube formation assay. 48 well plates were coated with a thin layer of collagen type I. Each well was seeded with 15,000 ECs and disks of alginate and alginate/PRP were placed in transwell membranes within the wells. The cultures were monitored over 2 days and were imaged at different time points. The formation of tubes was assessed using ImageJ Angiogenesis analyzer plugin. The number of loops/meshes, length of tubes and number of branch sites/nodes were determined.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 6 (San Diego, USA). Data were analyzed using one- and two-ways ANOVA multiple comparison. Statistically significant values are presented as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

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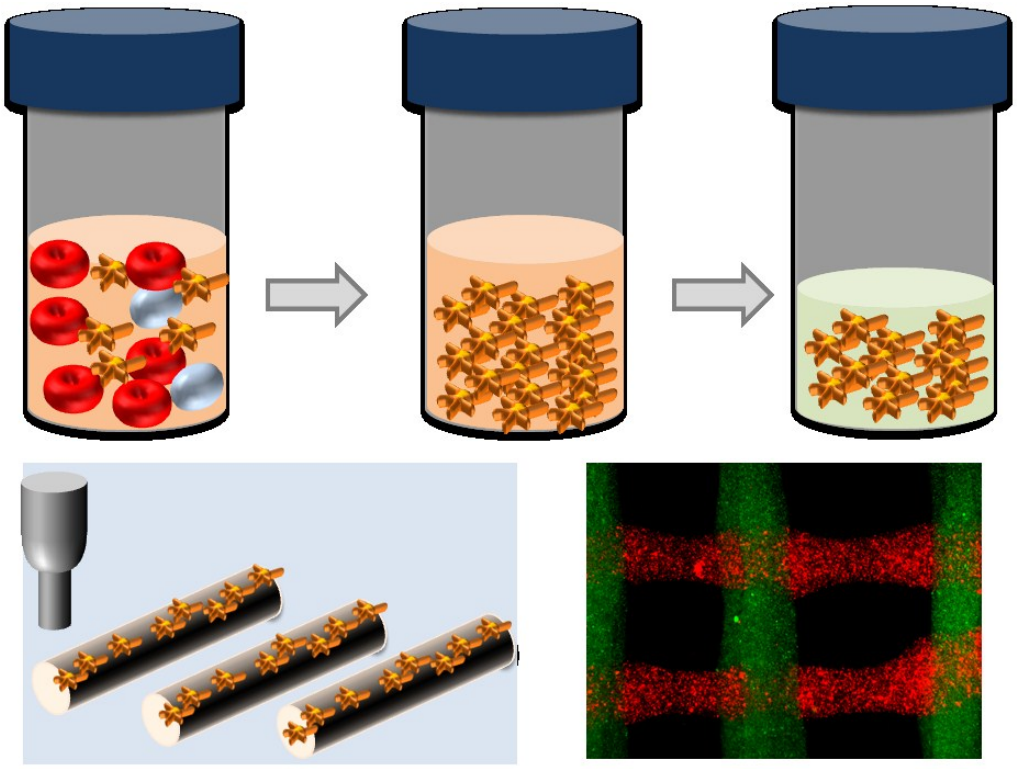

Incorporation of PRP with alginate-based bioinks enabled 3D bioprinting of scaffolds releasing autologous growth factors and proteins. The engineered bioinks can be printed using commercial bioprinters for various tissue engineering applications.

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