Subaqueous bioprinting: a novel strategy for fetal membrane repair with 7-axis robot-assisted minimally invasive surgery

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Abstract

Premature rupture of membranes (PROM), defined as breakage of the amniotic sac prior to delivery, complicates 2–4% of pregnancies, leads to one-third of preterm births, and increases the risk of perinatal mortality and morbidity in survivors. In vivo bioprinting enables the fabrication of three-dimensional (3D) structures directly for tissue repair or reconstruction in living animals but is challenging to perform in the subaqueous narrow space where fetal membranes (FMs) are located. This study reports a novel strategy consisting of an ultrafast photoresponsive hydrogel (1.5 s) and a 7-axis bioprinting robot to perform subaqueous in vivo bioprinting in a minimally invasive approach. Specially, a hydrogel patch with designed gel rivets is printed subaqueously for the therapeutic needs of PROM, and the patch displays robust tissue adhesion, favorable biocompatibility, mechanical properties resembling native tissues, and an appropriate sealing timescale to prolong pregnancy. Experiments performed in an in vitro uterus model and a mid-gestational rabbit model show a favorable sealing effect for PROM, which validates the potential of our subaqueous bioprinting approach for application. This study not only expands upon in vivo bioprinting techniques but also provides valuable insights for the treatment of PROM and other diseases involving tissue injury in clinical settings.

1. Introduction

Premature rupture of membranes (PROM), defined as the spontaneous rupture of membranes before 37 weeks of gestation and proven to be associated with 30–40% of preterm births, occurs in approximately 2–4% of all pregnancies and results in an increased risk of chorioamnionitis, perinatal death, and survivor morbidity\(^1,2\). The amnion is avascular and will not heal without intervention\(^3\), which has prompted several studies on treatments for PROM. Most attempts have focused on miscellaneous tissue sealants but have shown limited success\(^4,5,6,7\).

In vivo bioprinting is a new branch of 3D bioprinting that refers to the creation or repair of living tissues or organs by printing bioink directly on defective sites in a clinical setting\(^8\). It has been shown to be a promising approach for skin, cartilage, and skeletal muscle regeneration\(^9,10,11\), which has inspired PROM therapy. However, most bioink precursors would be attenuated in water-based fluids which would further affect the gelation process, making it challenging to perform in vivo bioprinting in a fluid environment such as amniotic sacs. Although tissue sealants gelled on wet surfaces have been explored with inspiration from mussels\(^12\) or barnacles\(^13\), these injectable materials do not possess properties important for printability, including shear-thinning and recovery behavior, and normally form an isotropic structure whose 3D shape and spatial organization cannot be controlled around the injection site.

In terms of bioprinting methods, recently reported in vivo bioprinting is limited to externally injured areas or sites exposed by surgery, and these methods require direct contact with the printing sites as well as at least three degrees of freedom (DoFs) of the bioprinting head in the X, Y and Z axes, making it difficult to use conventional bioprinting methods for intracorporal tissue repair. A noninvasive bioprinting strategy is able to address these difficulties but is still limited by restrictions related to the millimeter-scale size and
Minimally invasive surgery (MIS) is defined as accessing damaged tissues inside the body with minimal incisions to shorten wound healing time and reduce associated pain and risk of infection. Robot-assisted MIS has become more popular clinically in recent years due to its greater surgical precision, increased range of motion and improved dexterity. However, in vivo bioprinting in conjunction with MIS for tissue repair remains unexplored.

Herein, we propose a novel strategy of subaqueous in vivo bioprinting in a 7-axis robot-assisted minimally invasive approach and provide a potential treatment for PROM in clinical applications as shown in Fig. 1. The implementation of the strategy relies on: (i) a novel 7-axis bioprinting robot capable of intracorporal operations in an active method with proper control under the restrictions of MIS, mainly the remote center of motion (RCM) constraint, and (ii) an ultrafast photoresponsive GMPD hydrogel which is composed of methacrylated gelatin (GelMA) and poly (ethylene glycol) diacrylates (PEGDA), ensuring rapid gelation in a fluid environment. Specifically, with our technique, hydrogel patches with gel-rivet structures are printed, and they possess native tissue-resembled mechanical properties, robust tissue adhesion, favorable biocompatibility, and an appropriate timescale for prolonging gestation. The technique successfully demonstrates a favorable sealing effect for PROM both in an in vitro uterus model and a rabbit model at mid-gestation. This study presents the first concept of intracorporal subaqueous bioprinting and demonstrates its feasibility, which not only advances in vivo bioprinting technologies but also provides valuable insights for the clinical treatment of PROM and other diseases involving tissue injury.

2. Results

2.1 Preparation and characterization of GMPD hydrogels

To construct GMPD hydrogels, gel precursors of GelMA and PEGDA are prepared separately and then mixed with the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) in different ratios. The radicals induced by 405-nm light cleave the carbon-carbon bonds in GelMA and PEGDA, and then bond with each other to form stable interpenetrating networks (Fig. S3). Subaqueous printing is based on an appropriate initial viscosity as well as rapid crosslinking reactions so that the gel precursors are minimally diluted and dispersed, which are the obstacles that appeared in previous studies. Compressed samples of GelMA with different initial concentrations are prepared in air or water and then tested for compressive modulus. As shown in Fig. 2a, the modulus of hydrogel samples with concentrations above 15% shows no significant difference between preparations in air and water, while samples with lower concentrations are affected by their environment and are often unable to gel effectively in water. Through a rheological assay, gelation times are found to be 1.5 ± 0.6 s and 1.2 ± 0.5 s for 15% GelMA (abbreviated G15) and G20, respectively, with a light irradiance of 100 mW/cm², whereas at 30 W/cm² and 10 W/cm², complete crosslinking of G15 takes 5.2 ± 0.8 s and 11.9 ± 1.5 s, respectively (Fig. 2b, c). These longer gelation times may increase the risk of dilution.
Although photocrosslinking is a strong and irreversible reaction, the thermosensitive properties of pure GelMA hydrogels cause their decomposition at human body temperature\textsuperscript{16} and further lead to sealing failure for subsequent application in the treatment of PROM. The introduction of PEGDA significantly delays the thermal decomposition of the hydrogels, and the residual weight of the hydrogel samples with only 5\% PEGDA (w/v) increases from 60–90\% (p = 0.0007492, G15 compared to G15 with 5\% PEGDA (abbreviated G15P5)) after 14 days of immersion in 37\°C water (Fig. 2d). On the other hand, the modulus of the hydrogel increases significantly with the introduction of PEGDA, while the stress–strain curve shows a corresponding decrease in the maximum strain, appearing as the hydrogel samples become more brittle, which is undesirable in tissue sealing (Fig. 2e, Fig. S5). To optimize the ratio of GelMA and PEGDA, scanning electron microscopy (SEM) analysis is performed on hydrogels with different ratios. The SEM images reveal that the hydrogel with a lower PEGDA ratio has an interconnected-pore structure with pore diameters of 91.5 $\pm$ 14.4 $\mu$m, while with increasing PEGDA, the walls of the hydrogels become thicker and the porosity decreases (Fig. 2g, h), which provides a microscopic explanation for the increase in the compression modulus and the decrease in the swelling ratio (Fig. 2f). When the concentration of PEGDA exceeds 15\%, the porous structure disappears, and it is detrimental to the further application of the material.

Considering the resistance to thermal decomposition and the microstructure of the hydrogel, 5\% final concentration of PEGDA is chosen for the hydrogel, which not only maintains an over 90\% residual weight after immersion in Ringer’s solution at 37 \°C for 2 weeks but still possesses a porous structure and does not affect the toughness of the hydrogel (Fig. 2i). The modulus of G15P5 is 81.2 $\pm$ 12.7 kPa, which resembles the modulus of native fetal membranes (FMs)\textsuperscript{4}.

Rheological temperature sweep analysis of the hydrogels shows that the viscosity and modulus-temperature curves are essentially coincident and that the gel point temperature of different groups has no significant variation (Fig. 2j, k). This result indicates that the introduction of PEGDA has little impact on the thermosensitive properties of the gel precursors, which is important for subsequent regulation of the printability of the hydrogels.

### 2.2 Subaqueous printability evaluation of the GMPD hydrogel

Desired printability requires hydrogels with appropriate yield stress, shear thinning properties and rapid shear recovery behavior\textsuperscript{17}. Rotational rheological tests are performed on proportionally optimized hydrogels, and the results suggest that different groups of hydrogels all possess shear thinning properties (Fig. 3a). Many non-Newtonian bioinks demonstrate viscoelasticity and are accurately represented by the Herschel-Bulkley (H-B) model, expressed as $\tau = \tau_0 + K \cdot \gamma^n$ where $\tau_0$ denotes the yield stress, $n$ denotes the flow behavior index and $K$ represents the consistency index. The relationship between the shear rate and shear stress for G15, G15P5, and G15P10 are fitted with the H-B model (Fig. 3b), and all calculated H-B parameters are listed in Fig. 3c. This result indicates that the introduction
of PEGDA increases the overall yield stress and has an impact on the flow parameters. The preferred G15P5 has a yield stress of 68.13 Pa, which is acceptable for printability, as noted in previous studies\(^\text{18}\).

The flow behavior is critical for steady and continuous printing to achieve ideal fidelity. However, it is difficult to directly monitor the flow behavior within the nozzle; thus, the H-B fluid model is used here to analyze the flow of the hydrogel to predict the shear rate and velocity distribution during extrusion. At a flow rate of 0.25 mL/min, 80.4% of the radius \(R_0\) demonstrates plug flow where the hydrogel has merely internal shear stress that does not exceed the yield stress and behaves like a solid\(^\text{19}\). The hydrogel in the plug flow is extruded forward at the same velocity, while the shearing is confined to a narrow region along the extruder walls (Fig. 3f). In addition, the viscosity of G15P5 corresponding to the shear rate distribution ranges from 1.7 to 32.5 Pa·s and has been revealed to be suitable for extrusion-based bioprinting\(^\text{20}\).

These properties greatly improve the fidelity of the printed hydrogel filaments.

The GMPD hydrogel presents a typical shear-induced gel-fluid transition behavior and rapid self-healing. The strain sweep rheological assay on G15P5 exhibits a gel-fluid transition at 283% strain (Fig. 3d), and this transition is reversible. A cyclic shear applied to G15P5 at high (300%) and low (1%) strains shows that the hydrogel responds rapidly to shear and recovers immediately once the shear disappears (Fig. 3e). This shear thinning and self-healing can be repeated many times without sacrificing modulus.

MIS performed in the womb normally involves a fetoscope that can be abstracted as a rigid tubule with a length of 300 mm. In extrusion-based bioprinting within long needles, the state of the hydrogel may change due to long-term shear effects and may be detrimental to the printing fidelity. However, with the favorable printability of the GMPD hydrogel, it forms a robust, stable, and continuous hydrogel filament after extrusion from a 300 mm nozzle, and the length of the filament increases linearly during printing (Fig. 2g), even when the length extends to approximately 50 mm. These results indicate that the GMPD hydrogel possesses adequate printability and is suitable for subaqueous \textit{in vivo} bioprinting based on the long-range extrusion procedure. In addition, the velocity of the extruded filament \(V_\text{ink}\) and the nozzle feed velocity \(V_\text{noz}\) should be matched to obtain the desired printed pattern; when \(V_\text{ink}\) is lower than \(V_\text{noz}\), the continuity of the hydrogel filaments will not be guaranteed, and instead, the filaments will accumulate randomly, which is not conducive to 3D construction in water (Fig. S9a, b). As shown in Fig. 3h, filaments with diameters of 1.5 to 2.2 mm hold fidelity and 3D accumulation capacity, where the corresponding ranges of \(V_\text{ink}\) and \(V_\text{noz}\) are delineated.

For the treatment of PROM, compact hydrogel patches with uniform thickness are the most desirable 3D structures, and thus the fusion of filaments in the patches needs to be evaluated. Herein, the fusion index \(F_u\) is proposed and defined as \(F_u = L/(a + b)\) where \(L\) denotes the perimeter without a bottom edge, and \(a\) and \(b\) denote the height and length of the ideal gel patch, respectively. In overfusion where \(F_u < 1\), filaments aggregate and appear as a droplet under surface tension and go against multilayer printing, while in underfusion where \(F_u > 1\), insufficient fusion between filaments leads to imperfect patch surfaces and easily causes seal failure at the filament connections. Only under proper-fusion conditions can a dense and uniform hydrogel patch with \(F_u = 1\) be obtained (Fig. 3i). With the printing parameters guided
by the fusion index, a dense bilayer hydrogel patch with \( Fu = 0.9984 \) is printed subaqueously, and it maintained adequate mechanical properties to be picked up with tweezers (Fig. 3j, Supplementary Video 1).

### 2.3 Evaluation of robot-assisted bioprinting under MIS

MIS is a surgical approach in which operations are performed with long, narrow surgical tools that are held by a robotic arm and inserted into the patient through a small incision\(^{21}\). Surgical tools must pivot around these incisions which are called RCM constraints\(^{22}\) (Fig. 4a). Due to the redundancy associated with the additional DoF, the 7-axis robotic arm is capable of MIS at any spatial point as the RCM constraint actively, which provides greater flexibility compared to passive RCM approaches, including the da Vinci system produced by Intuitive Surgical, Inc\(^{23}\). Under the guidance of obstetricians, an end effector with a length of 300 mm and a diameter of 4 mm (similar to commercial fetoscopes) is designed, and a puncture mechanism, a syringe pump and optical bers are integrated (Fig. 4b) for subaqueous bioprinting.

The active RCM approach is implemented through the manipulator’s software controller; thus, infinite available RCM locations exist within the manipulator’s workspace that need further optimization to acquire improved motion accuracy and efficiency. Yoshikawa\(^{24}\) proposed a “manipulability measure”, and Salisbury\(^{25}\) proposed the condition number of Jacobian matrixes as a measure of kinematic quality, but they could evaluate only a specific configuration of the manipulator but not the kinematic performance within the entire workspace under one RCM constraint. Inspired by Stucco and Salcudean\(^{26}\), the global isotropy index (GII) is used to provide a measure of available RCM locations. The GII can be calculated as

\[
GII = \frac{\min_{X_1 \in \text{workspace}} \sigma_{\min}(X_1)}{\max_{X_2 \in \text{workspace}} \sigma_{\max}(X_2)}
\]

(2.1)

where \( \sigma_{\min}(X_1) \) is the smallest singular value of the Jacobian \( J \) at workspace position \( X_1 \), and \( \sigma_{\max}(X_2) \) is the largest singular value of the Jacobian \( J \) at workspace position \( X_2 \). A GII value of 1 indicates perfect isotropy, while a value of 0 indicates a singularity.

Figure 4c shows the GII distribution in the \( y = 0 \) plane. The best GII appears at \((0.45, 0, 0.26)\) (meters) with a value of 0.1192, and the white background indicates the RCM locations at which part or all of the workspace is unreachable. Different RCM locations correspond to different control properties. Trajectory tracking experiments are conducted at 17 RCM locations where the actual trajectories are captured with two orthogonally placed cameras and compared with the desired trajectories. The RMS deviation is displayed in Fig. 4c. The best RCM location possesses a minimal deviation of \( 0.177 \pm 0.011 \) mm, and the zigzag, star and circle trajectories performed at the best RCM location are listed in Fig. 4e, showing the
coincidence between theoretical and actual trajectories (Supplementary Video 3). The same zigzag trajectory is performed with a hand-held procedure under the RCM constraint (Fig. S11b), and the RMS deviation reaches 0.896 ± 0.172 mm, which is 5-fold larger than that of the robotic arm (Fig. 4d). Meanwhile, the instability of the velocity in trajectory tracking may lead to nonuniformity of the hydrogel filament diameter, which makes the constructed hydrogel patch poorly integrated and prone to seal failure (Fig. 4f, g).

The constraint of the printing angle (the angle between the nozzle and normal orientation of the fetal membrane) is important when determining the incision on the body. As revealed in our previous study[27], hydrogel filaments tend to slip off the substrate when the printing angle decreases. The FM and abdomen are abstracted as an ellipsoid and a plane, respectively, where the optimal incision is calculated by taking the printing angle as the optimization parameter, as shown in Fig. 4h.

An *in vitro* model is fabricated in accordance with the size of the human amniotic sac, and it is placed and punctured according to the calculated optimal RCM and incision location. Ringer’s solution at 37 °C is poured into the model to simulate amniotic fluid, and agarose gel is laid on the part to be printed to mimic soft tissue, such as the endometrium. Our bioprinting robot performs subaqueous bioprinting under RCM constraints, and the printed hydrogel patch is compact with a gel-rivet structure embedded in the agarose substrate (Fig. 4i, Supplementary Video 2). These results further demonstrate the feasibility and future application potential of GMPD hydrogels, the subaqueous printing process and the robot-assisted MIS approach.

### 2.4 Sealing evaluation of subaqueous bioprinting on *in vitro* models

To investigate the adhesion properties of the GMPD hydrogel, burst pressure tests and standard lap-shear tests are conducted. Figure 5a shows the schematic diagram, representative images and results of standard as well as modified burst pressure tests. The burst pressure obtained by the standard procedure solely relying on the adhesion of hydrogel is 37.0 ± 2.1 kPa, which is slightly lower than the 45.1 ± 4.2 kPa of the intact FM. However, the burst pressure recorded in the modified tests reaches 52.5 ± 7.2 kPa and shows no significant difference from that of the intact FM, indicating favorable sealing performance underwater. The same tests conducted on the intestinal mucosa of pigs presents similar results (Fig. S12d).

Lap shear tests are conducted according to the ASTM F2255-05 standard, and the shear strength between the hydrogel and the tissues is measured using two glass slides coated with human FM as the substrate. The shear strength of the crosslinked GMPD hydrogel reaches 33.2 ± 2.5 kPa which is higher than that of other commercial surgical sealants, including fibrin glue (18.2 ± 1.1 kPa) and PEG glue (21.9 ± 5.0 kPa) (Fig. 5b). To further improve the reliability of patch sealing, we propose a strategy in which the intermolecular forces of the material and mechanical forces of the specific structure combine to strengthen the adhesion of the hydrogel patch, namely the hydrogel rivets (Supplementary Video 4). With
the ability to fabricate structures with 3D shapes, the in vivo bioprinting approach prepares hydrogel patches with rivets that are embedded in the tissue and complement the interfacial adhesion of the hydrogel (Fig. S16).

In evaluating the adhesive strength of hydrogel rivets, hydrogel patches are printed on agarose gel with or without gel rivets, and two specially designed agarose substrates are connected with a patch. The tensile test results show that the adhesive strength of the patch with rivets improves approximately 6-fold in comparison to samples without rivets, from 2.43 ± 0.65 kPa to 14.53 ± 2.49 kPa (Fig. 5c, Supplementary Video 5). To simulate fluid sloshing in a real amniotic sac, we use a flow-controlled water stream to scour the patch with or without rivets, and the results indicate that the ability of patches with rivets to resist stream impact improves by approximately 7-fold (Fig. S12a, b, Supplementary Video 6).

An in vitro uterus model is established and punctured to mimic PROM for evaluating the sealing performance of various hydrogels. Different groups of hydrogels are printed subaqueously as sealants at the rupture site, and fluid leakage is recorded periodically. Figure 5d shows that hydrogel sealants without PEGDA are prone to seal failure due to partial dissolution at 37 °C, resulting in accelerated fluid leakage. However, the optimal G15P5 sealant maintains a leakage profile similar to that of the intact FM during the 21-day observation period (Supplementary Video 7), which validates the effectiveness of the subaqueous bioprinting sealing approach for treating PROM.

### 2.5 Sealing evaluation of subaqueous bioprinting on mid-gestation rabbit models

The biocompatibility of bioinks is essential for intracorporeal applications. We prepare cell culture plates coated with G15P5 hydrogel and culture rabbit amniotic epithelial cells on the surface of the hydrogel for 7 days. The cell viability assay indicates that the cells maintain over 90% viability in the 7-day culture in both the experimental and control groups (normal 2D culture) (Fig. S13a), and the representative images of Calcein AM/propidium iodide immunofluorescent staining shows that the cells could extend to spindle or diamond shapes in the appearance of the G15P5 hydrogel (Fig. S13c). The results of the CCK-8 assay in Fig. S13b also show that the cells proliferate well in the experimental groups.

Pregnant rabbits at mid-gestation are considered a suitable model to study FM defects and to validate PROM sealing techniques and are therefore selected for intracorporeal research and evaluation. The average duration of a rabbit's pregnancy is 32 days, with litters of approximately eight offspring. The amniotic sac reaches a size of 4–5 cm at a gestational age (GA) of 22 days, which is compatible with FM repair tests. According to previous studies, one to two amniotic sacs in one rabbit are selected as the experimental group and positive control group (Fig. 6a). Sacs in the experimental group are punctured and sealed with an I-shaped hydrogel patch that could be embedded at the incision, whereas sacs in the positive control group are simply punctured. At a GA of 31 days, the rabbits are euthanized for a second hysterotomy to evaluate the sealing performance (Fig. 6b). One rabbit died due to surgical infection, while the other eight survived without herniation of the fetus into the maternal abdomen at sacrifice. After
myometrial dissection and opening of the gestational sac, no intraamniotic adhesions or amniotic bands are observed. Further data on the outcome are presented in Fig. 6d.

The fetal survival rate is 72.7% in the sealing group, similar to 81.3% in the native control group. Among them, 10 out of 11 hydrogel patches could be traced, and 8 out of 10 remained stably mounted on the FM, and these eight sacs remain full of amniotic fluid. The remaining two patches separated from the FM and are found freely in the uterus. In the positive control group, amniotic fluids almost vanish in all sacs, and only one fetus survived. The average weight of fetuses in the experimental group is $33.2 \pm 4.7$ g compared to $8.6 \pm 3.1$ g in the positive control group, indicating that fetal development stops shortly after FM puncture and that fetuses are partially resorbed. Both gel sealant and FM could be identified from sealed tissues processed for histological evaluation. From HE-stained sections, it could be seen that the GMPD hydrogel is tightly attached to FM. At larger magnifications, the GMPD hydrogel is observed to be well integrated with FM, completing the restoration of membrane integrity and even exhibiting re-epithelialization. (Fig. 6c).

In subcutaneous implantation experiments in rats, the tissue around the implanted GMPD hydrogel undergoes further histological and immunofluorescence assays at 7, 14 and 21 days after implantation to verify immunogenicity. When a hydrogel is implanted, the body’s immune system recognizes such implants as foreign and encapsulates them in dense collagen. The morphology and thickness of the collagen capsule can be considered metrics of immunogenicity. Masson’s trichrome staining of the implanted tissue after three weeks shows that the collagen capsule is discontinuous and not compact with a thickness of ~ 50 µm, indicating that the hydrogel does not elicit a severe substantial foreign-body reaction (Fig. 6e). HE staining on day 21 reveals the presence of the G15P5 hydrogel and shows favorable integration with the native tissue (Fig. 6f). In addition, immunofluorescence staining for CD68 (Fig. 6g) shows few macrophages (stained in green) after 21 days. Together, these assays indicate that GMPD hydrogels present low immunogenicity and favorable biocompatibility that can be considered an ideal biomaterial for intracorporal applications.

3. Discussion

The FM acts as an essential barrier to protect the fetus from the external environment. Once PROM occurs, the leakage of amniotic fluid and the access of external bacteria may lead to the lack of a necessary fluid environment for fetal development and further result in hypoplasia and intrauterine infection. PROM affects approximately 2–4% of pregnancies, and with the very large number of births, millions of families with newborns will be affected to various degrees. Although a large number of PROM treatments have been attempted, including mixtures of maternal platelets and fibrin cryoprecipitate, collagen/gelatin plugs, synthetic polymer sealants and laser welding approaches, these have met with limited success, and no clear pathway to a clinically viable solution has emerged after decades of research. Although in vivo bioprinting has been proposed only recently, it has been proven to be a potential approach for the treatment of tissue injuries due to its immediacy, convenience and
functionality. However, the inside of amniotic sacs can be considered as the most challenging location to attempt bioprinting in the human body due to its subaqueous environment and limited operating space, and it can only be accessed in a minimally invasive method. Hence, the preliminary advances in *in vivo* bioprinting inside the amniotic sacs not only provide a new strategy for PROM, but also promote the development of *in vivo* bioprinting techniques and facilitate the treatment of a wider range of tissue injuries.

As Winkler et al. contended, an ideal membrane sealing material would have mechanical properties similar to those of FMs, subaqueous formability, and nonimmunogenicity, while it would maintain adhesion over an appropriate timescale to prolong gestation (3–4 weeks). Some materials with strong adhesion on wet surfaces have been recently reported, such as barnacle-inspired paste by Yuk and mussel glue by Kivelio et al. Even so, creating structures from biomaterials with 3D structures is considered more significant, not only for stronger sealing but also to match defective tissues for subsequent tissue repair and regeneration. Printing 3D structures in liquids is not unprecedented. Broadly speaking, gel baths such as the FRESH technique and oil bath-based printing methods can be classified as printing in liquids. Among them, the high density or molecular polarity of these supportive baths enables sound formability. However, printing in a low-viscosity hydrated environment such as water not only fails to assist the fidelity of structures but also has adverse impacts on gel formation due to the dilution of the gel precursors. The GMPD hydrogel reported here possesses ideal subaqueous printability, inferior immunogenicity and suitable mechanical properties, and it extends the applications of bath printing techniques.

In *vivo* surgical operations, especially in the field of obstetrics, basically rely on obstetricians to perform treatments with a hand-held fetoscope, including amniotic fluid examination and laser ablation for the treatment of twin-twin transfusion syndrome (TTTS), while merely robot-assisted fetal surgeries were reported in decades. Several studies have indicated that there is uncertainty in manual surgeries, which agrees with our study results, and would affect the success rate of fetal surgeries and postoperative recovery. A recent review reported that the number of times surgical instruments are inserted into the uterine cavity and the duration of the surgery are critical to successful fetal surgeries. Therefore, the primary goal of repair mechanisms is to minimize the duration and invasiveness of surgical intervention, which aligns with our approach of printing reliable hydrogel patches onto the FM with high precision under minimal invasiveness. In addition, the tiny size of natural FM ruptures, generally 2–3 mm in diameter, makes manual operation more difficult. Therefore, a robot-assisted approach is more suitable in this scenario.

The 7-axis serial robot used in this study has redundant properties (possessing 7-DoF, more than 3-DoF for the RCM constraint and 3-DoF for surgical tasks), which is capable of performing MIS under the RCM constraint in an active approach. Although more research on robotic bioprinting has been reported recently, the conventional 6-axis manipulators used in previous studies do not have task redundancy and cannot perform the above operations. In addition to fulfilling the RCM constraint, redundancy can
also be exploited to achieve other additional tasks, such as obstacle avoidance, human-like behavior, and manipulability optimization in a human–machine cooperation scenario, which further advances this therapeutic technique for tissue repair on the interior or exterior of the human body. In terms of manipulability, flexibility and cost effectiveness, the active RCM strategy represented by the 7-DoF manipulator is advantageous, with an optimal manipulability of 0.1192 for our serial bioprinter in comparison with 0.03–0.07 for the da Vinci. Even so, there is still no MIS active surgical robot in use in clinical settings because of the unguaranteed RCM point in the event of controller failure, and most correlational studies are stuck in the early and nonclinical stages. To further promote the clinical application of active surgical robots, advances in various fields, such as safety control of robots, are needed.

Statistically, most cases of PROM occur at a GA of 25–32 weeks, and approximately 60% of premature deliveries occur within the first week after PROM; dysontogenetic newborns from these cases are extremely difficult to keep alive. A study conducted by the Lancet indicated that the survival rate of extremely preterm infants, defined as being delivered before 28 weeks, was only 17%-41% and was accompanied by a disability rate of approximately 30%. As long as the GA can be extended beyond 28 weeks, survival rate can be increased to over 80% with medical treatment, which guides us to set the duration for sealing experiments in the \textit{in vitro} model to 21 days. Although the intact FM shows leakage of the contained fluid, probably due to the loss of bioactivity, the leakage curve of the hydrogel sealing groups over 21 days is consistent with the intact FM. In addition, the residual weight of the G15P5 hydrogel remains above 75% after 40 days of immersion in 37°C water, which implies that the actual sealing duration of the GMPD hydrogel \textit{in vivo} might be longer than 21 days, achieving reliable long-term sealing.

In the animal experiments for PROM, the rabbit model is chosen due to its affordable housing demands and large litter size. However, the size and shape of the uterus and FM are much different from those in human or nonhuman primates, which prevents the robot-assisted MIS and hydrogel rivets from being embedded. Due to the shorter gestation of rabbits, only nine days of intracorporal sealing are performed. Nevertheless, the \textit{in vivo} bioprinting approach still reveals the sealing integrity of FM and promising re-epithelialization. Other animal models that could be further studied on robot-assisted FM healing are the sheep or primate model with a gestation of 145–150 or 165 days, respectively.

As a typical medico-engineering collaborative study, future studies are required to investigate many aspects, including: (i) a photocrosslinking approach with long wavelengths to avoid unknown impacts on the fetal development; (ii) robust controller design for further application of active robot-assisted MIS; (iii) development of machine vision for confirmation of rupture site locations; and (iv) the effectiveness of this system in preclinical large-animal studies and human clinical trials. Although in the early stage, the subaqueous \textit{in vivo} bioprinting approach for tissue sealing offers a promising option for blocking leakage of amniotic fluid, prolonging the GA and improving the survival rate of premature infants in cases of PROM. We envision that this novel subaqueous printing strategy will not only provide an effective
approach for PROM but also offer valuable insights for the treatment of other tissue injury diseases in clinical settings.

4. Method

4.1 Preparation of GMPD hydrogel

Methacrylate gelatin (GelMA), poly (ethylene glycol) diacylates (PEGDA), and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) were purchased from Engineering for life (EFL Inc., China). And the two gel precursors were prepared according to the instructions separately. Briefly, quantitative lyophilized GelMA and PEGDA were dissolved in phosphatic buffer solutions (PBS) with 0.5% LAP at 40 °C, and filter-sterilized through a 0.22 µm PES syringe filter (Membrane Solutions). Hydrogel precursor solutions of different concentrations were obtained, and were mixed intensively according to different ratios. In this paper, the GmPn bioink indicates that GelMA and PEGDA concentrations are m% (w/v) and n% (w/v) respectively.

4.2 Development and control of the 7-axis bioprinting robot

The end effector was designed based on the size of existing fetoscopes, with a coaxial structure and a diameter of 4 mm as well as a length of 300 mm. The outer side of end effector is optical bers, which are used for light conduction, while the inner side is the printing needle solidified with a screw motor to perform linear movement. The long needle is connected to a micro-syringe pump via Teflon tubing for quantitative control of extrusion. The end effector was integrated at the end of the 7-axis manipulator (Franka Emika Panda, Germany), and the tool central point (TCP) calibration was performed.

The trajectory of the end effector was automatically generated by the path generation algorithm (see supplementary information) according to the position and morphology of rupture site. The optimal puncture site, or is called the optimal RCM location, was obtained taking the minimum printing angle as the optimized parameter. Once the end-effector position and RCM location were determined, the required end-effector axis $k_{ee}$ can be trivially computed as

$$\hat{k}_{ee} = o_{ee} - o_{rcm}$$

Joint angle of the manipulator corresponding to the trajectory is obtained by an analytical inverse kinematics (IK) solver (see supplementary information). Furthermore, the manipulator was controlled using its built-in position-mode controller, and communication between the Ubuntu 18.04 and the robot was achieved using the Franka Control Interface with a control period of 1ms.

4.3 Evaluation of RCM location

Two design parameters were used that the x- and z- of RCM location, and discretized into an 11 × 11 point grid in the y = 0 plane. The y-coordinate was not varied for the manipulator can pivot on its first axis,
rending other candidate RCM locations equivalent to those considered. For each candidate RCM location, the corresponding workspace was abstracted as a 100 × 100 × 100 mm cube, centered on a point 100 mm directly below the RCM point. And a 3D grid of 10 × 10 × 10 discrete workspace points was chosen inside the cube. Inside the workspace, the inverse kinematics corresponding to each test point was obtained through our IK solver, then the GII of the workspace can be calculated by Eq. 2.1 to evaluate the isotropy of candidate RCM points. The manipulability of conventional 6-DoF manipulator was also analyzed through this approach and yoshikawa’s manipulability.

The practical trajectory of end effector was centered in the fields and captured via two orthogonally positioned cameras (Lena3D, China) with a spatial resolution of ~ 60 µm per pixel. The cameras were calibrated using the camera calibration toolbox in MATLAB to provide the space coordinates, and to calculate the velocity of trajectory through the frame rate. The zigzag, star and circle trajectory following were performed under RCM constraints for each RCM location. Each trajectory was repeated five times respectively and the RMS deviation is obtained by comparing with the theoretical trajectory.

### 4.4 Computation modeling of extrusion

The Herschel-Bulkley computational model was used to simulate the rheological behavior of GMPD hydrogel and predict the shear stress and velocity distribution during extrusion-based bioprinting. The derivation of computation model is in the supplementary material. Briefly, for laminar, isothermal flow of an incompressible fluid with no-slip boundary conditions, the shear stress of the inner wall of needle $\tau_{wall}$ can be calculated from Eq. 4.2, i.e.,

$$Q = \pi R^3 \left( \frac{n}{n + 1} \right) \left( \frac{\tau_{wall}}{K} \right)^{\frac{1}{n}} \left( 1 - \frac{\tau_0}{\tau_{wall}} \right)^{\frac{n+1}{n}} \left( 1 - \frac{2n}{(2n+1)(3n+1)} \left( 1 - \frac{\tau_0}{\tau_{wall}} \right) \left( \frac{\tau_0}{\tau_{wall}} n + 2n + 1 \right) \right)$$

where $Q$ stands for the flow rate of extrusion, $\tau_0$ denotes the yield stress, $R$ is the inner radius of needle, $n$ is the flow index and $K$ is the flow consistency index.

Once $\tau_{wall}$ was determined, the shear rate $\gamma$ and velocity $u$ profiles could be calculated for all positions within the flow from equations 4.3 and 4.4, i.e.,
\[ \gamma = \begin{cases} 0, & 0 \leq r \leq R_0 \\ \left( \frac{\tau_{\text{wall}}}{K} \right)^{\frac{1}{n}} \left( \frac{r}{R} - \frac{\tau_0}{\tau_{\text{wall}}} \right) \left( 1 - \frac{\tau_0}{\tau_{\text{wall}}} \right)^{\frac{n+1}{n}}, & R_0 \leq r \leq R \end{cases} \]  \tag{4.3}

\[ u = \begin{cases} R \left( \frac{n}{n+1} \right) \left( \frac{\tau_{\text{wall}}}{K} \right)^{\frac{1}{n}} \left( 1 - \frac{\tau_0}{\tau_{\text{wall}}} \right)^{\frac{n+1}{n}} - \left( \frac{r}{R} - \frac{\tau_0}{\tau_{\text{wall}}} \right)^{\frac{n+1}{n}}, & R_0 \leq r \leq R \end{cases} \]  \tag{4.4}

where \( R_0 \) stands for the radius of plug flow region.

The equations above were solved and plotted in MATLAB (MathWorks, MA) to reveal the extrusion distribution inside the needle.

### 4.5 Burst pressure test

The burst pressure test was performed according to ASTM F2392-04 with minor modifications. In the burst adhesion test, freshly collected human FM were fixed to the measurement device linked to an air pump. A 2 mm incision was made on the FM and the membrane surface was kept wet. Then, 500 µL G15P5 hydrogel precursor was injected and \textit{in situ} crosslinked simultaneously as a patch to seal the puncture site. The peak pressure before pressure loss was considered the burst pressure to evaluate the tissue-adhesive properties. After that, turned the measuring device upside down and filled it with Ringer's solution at 37 \(^\circ\)C. Once the incision was created on the FM, fluid contained leaks through the puncture site, and 500 µL G15P5 hydrogel precursor was immediately printed subaqueously to seal the leakage. Burst pressure peaks were detected by increasing the hydraulic pressure above, and it reflects the sealing ability to sustain hydraulic pressure. All measurements were repeated three times and freshly harvested pig intestinal mucosa was subjected to the same test.

### 4.6 Lap shear test

The lap shear test was conducted according to ASTM F2255-05 for lap strength property of tissues adhesion. The freshly collected human FM was cut in to 2.5 cm × 2.5 cm and attached to glass slides with cyanoacrylate glue. Then, 200 µL of gel precursors was injected onto the surfaces of FM and irradiated with a 405 nm LED (100 mW/cm\(^2\)) immediately for polymerization. The two glass slides were fixed into the electro force 5500 system (BOSE electro force systems group, Friedrichsdorf, Germany) for shear testing by tensile loading with a strain rate of 1 mm/min. The tissue adhesive strength of the hydrogel was calculated according to F/A, where F is the pulling stress and A is the adhesive area. All
measurements were repeated three times and Fibrin Glue (Shanghai RAAS Blood Products Co., Ltd, China), HAMA gel (Engineering for life Inc, China) and PEG glue (Medprin Regenerative Medical Technologies Co., Ltd, China) were tested using the same parameters and conditions.

4.7 Adhesive strength tests of hydrogel patch

The adhesive strength of the hydrogel patch was evaluated through a modified approach according to ASTM F2458. 2% agarose gel was cast in a petri dish and trimmed as a substrate to mimic the soft tissues. Hydrogel patches with or without gel rivets were printed on the substrates to connect them. The two substrates were placed beside each other in close proximity and hydrogel patches were printed on them with or without gel-rivets to connect the two substrates. After that, tensile tests were carried out on the samples with the electro force 5500 system (BOSE electro force systems group, Friedrichsdorf, Germany), and the maximum force at failure was recorded and was divided by the bonding area to find the adhesive strength.

4.8 Evaluation of the sealing effect in the in vitro uterus model

The in vitro uterine model was established with a cylinder modified from syringe, fresh human FM and filleted poultry breasts. The cylinder was injected with Ringer's solution (Yuanye biotechnology Co., Ltd, China) according to human physiological pressure inside ammonic vesicles (normally 20 mL), and FM was wrapped underneath and the poultry breast was secured on the outside to simulate the anatomical structure of FM and uterine wall musculature. A perforation with a diameter of 2 mm was created on the FM, where the Ringer's solution leaked from the cylinder as PROM. After that, different groups of hydrogels were printed subaqueously as sealants for the incisions. The upper end of the cylinder was sealed with plastic wrap to prevent the evaporation of internal fluids and the inferior FM and poultry breast were soaked in PBS to keep moist. The overall setup was placed in an incubator at 37 °C and the leakage of fluids was recorded at regular intervals.

4.9 Rat’s subcutaneous implantation

Six male 12-week-old Sprague Dawley rats weighing approximately 250 g were randomly assigned GMPD hydrogel implantation group and control group. Rats were anesthetized through isoflurane inhalation (RWD Life Science, Shenzhen, China). The dorsal hair was shaved using an electric clipper, and the naked skin areas were cleaned with 70% ethanol and disinfected with povidone-iodine solution. An incision was created along the dorsolateral aspect and a subcutaneous pocket was created through the blunt dissection of dermal tissue from the underlying muscle layer. G15P5 hydrogel precursor was injected into the subcutaneous pockets and in situ crosslinked immediately with at least two samples implanted per animal. For control groups, the subcutaneous pockets were injected with 200 µL of PBS. The incisions were then closed with nylon sutures (Ethilon, Ethicon). Rats were sacrificed at 7, 14, 21 days after implantation, and the implant sites with native tissues were excised and fixed for subsequent histology and immunohistochemistry studies. All the animal experiments were complied with the guidelines of the Tianjin Medical Experimental Animal Care, and animal protocols were approved by the Institutional...
Animal Care and Use Committee of Yi Shengyuan Gene Technology (Tianjin) Co., Ltd. (protocol number YSY-DWLL-2022058)

4.10 Rabbit model of fetal membrane defects

Nine time-dates pregnant New Zealand rabbits were operated at 22 days of gestation. The rabbits were premedicated with intramuscular injection of ketamine 25 mg/kg (Macklin, Shanghai, China) and xylazine 6 mg/kg (Macklin, Shanghai, China). followed by anesthesia with isoflurane (1-1.5%) in oxygen at 1.5 L/min (RWD Life Science, Shenzhen, China). Preoperative medroxyprogesterone acetate 9 mg/kg (Rhawn, Shanghai, China) was administered intramuscularly for tocolysis, and Penicillin G 300,000 units IM (Macklin, Shanghai, China) as prophylactic antibiotic. The rabbits were placed in a supine position and then shaved under continuous aspiration. After disinfection with povidone iodine, rabbits were draped with sterile fields so that interventions on the uterus and the membranes were performed under sterile conditions. Following preparation and stabilization of a surgical plane of anesthesia, an 8 cm lower midline laparotomy was performed and the uterus was exposed through the incision. Gestational sacs were counted and the uterine sacs near the ovary were chosen as experimental groups or positive control groups. In experimental groups, a segment of the uterus corresponding to a single fetus was exposed and an amnion defect was created with a 16-gauge (1.9 mm) needle. After that, a G15P5 gel patch with I-shape was printed at the defect to seal the puncture site (n = 11). While in positive controls, the amnion sacs were punctured but without sealing (n = 11) and the remaining sacs (n = 32) served as negative controls. The myometrial layers and the abdomen were closed by polypropylene 6 − 0 (Prolene, Ethicon) sutures. After reposition of the uterus, the abdomen was closed in layers with polyglactin 2 − 0 (Vicryl, Ethicon) for the fascia and intracutaneous nylon 3 − 0 (Ethilon, Ethicon) for the skin. After recovery in the operating facility, the animals were returned to their cages and allowed free access to chow and water.

At 31 days of gestation, rabbits were anesthetized with isoflurane (1-1.5%) in oxygen at 1.5 L/min to undergo a second-look hysterotomy. A myometrial incision was made, followed by gentle dissection with microsurgical instruments to expose both punctured and control sacs. The survival rate was recorded by needle aspiration, and the presence of gel patch and AF were recorded. The integrity of the punctured sacs was tested by injecting saline solution dyed with methylene blue using a 26-gauna needle through the membrane into the sacs on the opposite side of the defect. The gel sealant with surroundings native tissues (including fetal membrane and uterus) were excised and fixed in phosphate-buffered 4% paraformaldehyde solution for further histology studies. After all fetuses were weighted, the rabbits were euthanized with a T61 injection (Yanchen, China). All the animal experiments were complied with the guidelines of the Tianjin Medical Experimental Animal Care, and animal protocols were approved by the Institutional Animal Care and Use Committee of Yi Shengyuan Gene Technology (Tianjin) Co., Ltd. (protocol number YSY-DWLL-2022057)

4.11 Histology and Immunofluorescence
Histology and immunofluorescence studies were performed according to previous studies. Briefly, the tissue samples from rat subcutaneous implantation and rabbit FM sealing were fixed in 4% paraformaldehyde overnight at 4°C. After fixing, samples were washed three times for 5 min with 70% ethanol. Washed samples were then processed, and embedded in paraffin. 4 µm thick paraffin sections were cut and mounted onto slides. The tissue fusion was obtained by staining with Hematoxylin-Eosin (H&E), which stains nuclei dark purple/blue to black and stains cytoplasm pink. The fibrous capsule formation was stained with Masson's trichrome stain, which stains collagen blue, cytoplasm red and nuclei black. Immunofluorescence staining was carried out using CD68 primary antibody (Sigma, ab283654), Goat Anti-Rabbit IgG H&L (Sigma, ab15008) and DAPI (Sigma, D9542) with the subcutaneous tissue samples for inflammatory response, where macrophagocyte was stained green and nuclei were stained blue. Images were taken and analyzed under a laser scanning confocal microscope (Zeiss, Germany).

For each sample, three different fields of view were captured and analyzed in ImageJ for the thickness of fibrous capsules in Masson's trichrome stain and the number of macrophages in the immunofluorescence staining.

### 4.12 Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using either an unpaired student t-test for comparison between two groups or one-way ANOVA, followed by Dunnett’s multiple comparisons test, for multiple comparison tests. Differences were considered significant when \( P \leq 0.05 \).

### Declarations

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Figures
Figure 1

Schematic diagram of intracorporeal subaqueous bioprinting. **a**, The 7-axis bioprinting robot performs *in vivo* bioprinting through MIS approach on a pregnant women to treat PROM. **b**, Schematic illustration of the photo-crosslinking of GMPD hydrogels after exposure to blue light. **c**, Printing end effector performs subaqueous bioprinting for gel patch in the amniotic sac to seal the FM rupture. **d**, Flowchart of printing gel rivets inside the endometrium.
Figure 2

**Preparation and characterization of GMPD hydrogel.**

**a,** Compression modulus of GelMA hydrogels at different concentrations in liquid and in air. **b,** Photo-rheological results of G15 under blue light exposure with different irradiance. **c,** Gelation time of G15 and G20 under different blue light irradiance. **d,** Weight loss curves of G15 with different PEGDA ratios in a 37°C-water bath environment. **e-f,** Compression modulus and swelling ratio of different concentrations of GMPD hydrogel standard samples. **g,**
Representative SEM images of lyophilized GMPD hydrogel samples with different concentrations. (Scale bar: 100 μm). h. Porosity of GMPD hydrogel samples with different concentrations acquired through SEM images. i. Tradeoff between residual weight in day 14 and maximum strain of GMPD hydrogels with different concentrations. j. Temperature sweep assays conducted on G15 with different PEGDA ratios. k. Gelation temperature of G15 and G20 with various PEGDA ratios. (n = 3 independent samples) (ns: P > 0.05; *: 0.01 < P < 0.05; **: 0.001 < P < 0.01; ***: P < 0.001)
Subaqueous printability evaluation of GMPD hydrogel. a-b, viscosity and shear stress versus shear rate curves of G15 with different PEGDA ratios and fitted with Herschel-Bulkley model in steady shear experiments. c, H-B model parameters fitted according to shear stress-shear rate curves. (n = 3 independent samples) d, Strain sweeps of G15P5 hydrogel. e, Reversible gel-sol transition and self-healing of G15P5 hydrogel upon cyclic shearing at 0.1 % and 300% strains. f, Velocity and shear rate distribution in the nozzle's cross section of G15P5 hydrogel, where R_0 indicates the radius of plug flow. g, The steady flow of G15P5 hydrogel out of the nozzle. h, Diameter of extruded filament with different V_{noz} and V_{ink}. i, Evaluation of printed filaments fusion conditions with schematic diagrams, side view photographs as well as abstracted boundaries. j, Photograph of a two-layer gel patch printed subaqueously, and the patch possesses ideal mechanical properties that can be picked up by a tweezer. (Scale bar: 1 cm).
Figure 4

**Evaluation of robot-assisted bioprinting under MIS.** a, Schematic diagram of end effector moves under RCM constraint. b, Photograph of the 7-axis bioprinting robot, the screw motor for linear movement and the section views of the end effector showing the distribution of nozzle and optical fibers. (Scale bar: 2 mm). c, Evaluation of RCM location through GII index and the measured motion accuracy taking each spot as RCM constraint. d, RMS accuracy and RMS velocity deviation of bioprinting robot and hand-held
printing end effector. \( n = 3 \) independent tests) \((*: 0.01 < P < 0.05)\) e, Top view and side view of the orthogonal cameras to capture the actual trajectory of the end effector. (Robot-held, taking (0.45, 0, 0.26) as the RCM location, and the red line indicates the actual trajectory and the blue line indicates the desired trajectory. (Scale bar: 5 mm). f-g, Actual trajectory and instantaneous velocity of zigzag path between bioprinting robot and hand-held end effector. h, Optimization of incision location taking the printing angle as the optimization parameter. i, The 7-axis bioprinting robot performed subaqueous bioprinting at 37°C environment through a minimal incision and printed a gel patch embedded in the agarose substrate. (Scale bar: 1 cm).
Figure 5

Sealing evaluation of subaqueous bioprinting on *in vitro* models. **a,** Schematic diagram, results and photographs of various burst pressure test. **b,** Schematic diagram, results and photographs of lap shear tests for HAMA gel, Fibrin glue, PEG glue and G15P5 hydrogel. **c,** Schematic diagram and results for adhesive strength tests with gel patch with or without gel rivets. (Scale bar: 1 cm) **d,** Establishment of the *in vitro* uterus model and the sealing results of different hydrogels performed within the model. (*n* = 3 independent tests) (ns: *P* > 0.05; *: 0.01 < *P* < 0.05; **: 0.001 < *P* < 0.01; ***: *P* < 0.001)
Figure 6

Sealing evaluation of subaqueous bioprinting on animal models. **a**, The schematic diagram of the PROM sealing experiment performed on mid-gestation rabbits models. **b**, The main process in PROM sealing experiment, which contains PROM modeling and printing I-shape gel-patch for sealing at the gestation age of 22 days (Scale bar: 3 mm), and a second-look hysterotomy to evaluate the sealing performance at the gestation age of 31 days. (Scale bar: 1 mm). **c**, Representative HE staining of sealing sites and their
surrounding tissues and same areas in positive control and negative control groups. (Scale bar is 200 μm for the above three staining and 50 μm for the magnified view). d, Statistical data of sealing performance of GMPD sealing group and control groups. e, Representative Masson's trichrome staining of collagen capsule (pointed out by blue arrows) after 21 days of subcutaneous implantation. (Scale bar: 200 μm). f, Representative HE staining of G15P5 hydrogel's subcutaneous implantation after 21 days, showing the integration between hydrogels and native tissues. (Scale bar: 100 μm). g, Representative immunofluorescence staining of CD68 and DAPI of G15P5 hydrogel's subcutaneous implantation after 21 days, where green denotes the macrophages and blue denotes the cell nucleus. (Scale bar: 100 μm).

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