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 ≈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. Accessing the amniotic sac through a fetoscope in a minimally invasive approach is a common clinical operation for the diagnosis of PROM, making delivery of plugging materials through the fetoscope a promising therapeutic strategy.[4–6] However, due to the difficulties of precise injury-targeted operation and insufficient intracorporeal stability of materials in liquid surroundings, the plugging strategies have not advanced into clinical practice.[7]

In situ bioprinting is a new branch of three dimensions (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] and it has been shown to be encouraging in the repair of superficial tissues such as skin, cartilage, and skeletal muscle.[9–11] Nevertheless, it is still challenging for internal tissues repair through in situ bioprinting, such as PROM treatment. In a fluid environment inside amniotic sacs, most bioink precursors would be attenuated in the water-based fluids that would further affect the gelation process. 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, which prevents the printing of 3D structures in a low-viscosity hydrated environment.

In terms of bioprinting methods, recently reported in situ bioprinting is limited to externally injured areas or sites exposed by surgery. Minimally invasive bioprinting is an emerging strategy to address these difficulties, but is still restricted by the millimeter-scale size and depth of hydrogel cross-linking[11,14] or limited workspace.[15] The rupture of fetal membranes in human is ≈2–6 mm in size.[16,17] During the minimally invasive operation, the incision point acts as a fulcrum and constitutes a lever with the fetoscope. The scaling of the motion places an extreme demand on manual operation for
the surgeon, especially dealing with such tiny ruptures. Hence, the robot-assisted minimally invasive surgery appears necessary clinically due to its greater precision, increased range of motion and improved dexterity.\[18\] However, in situ bioprinting in conjunction with robot-assisted minimally invasive surgery for internal tissues repair remains unexplored.

Herein, we propose a novel strategy of subaqueous in situ bioprinting in a 7-axis robot-assisted minimally invasive approach and provide a potential treatment for PROM in clinical applications as shown in Figure 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 minimally invasive surgery, mainly the remote center of motion (RCM) constraint, and ii) an ultrafast photo-responsive GMPD hydrogel that 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 the ex vivo uterus models and mid-gestational rabbit models. This study presents the first concept of intracorporal subaqueous bioprinting and demonstrates its feasibility through a novel 7-axis bioprinting robot, which not only advances in situ 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 (Figure S3, Supporting Information). Subaqueous printing is based on an appropriate initial viscosity as well as rapid cross-linking 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 Figure 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 and 1.2 ± 0.5 s for 15% GelMA (abbreviated G15) and G20, respectively, with a light irradiance of 100 mW cm\(^{-2}\) whereas at 30 and 10 W cm\(^{-2}\) complete cross-linking of G15 takes 5.2 ± 0.8 and 11.9 ± 1.5 s, respectively (Figure 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\[19\] and
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)
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% to 90% (p = 0.0007492, G15 compared to G15 with 5% PEGDA (abbreviated G15P5)) after 14 days of immersion in 37 °C water (Figure 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 (Figure 2e; Figure S5, Supporting Information). 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 ± 14.4 μm, while with increasing PEGDA, the walls of the hydrogels become thicker and the porosity decreases (Figure 2g,h), which provides a microscopic explanation for the increase in the compression modulus and the decrease in the swelling ratio (Figure 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 possess a porous structure and does not affect the toughness of the hydrogel (Figure 2i). The modulus of G15P5 is 81.2 ± 12.7 kPa, which resembles the modulus of native fetal membranes.7

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 (Figure 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.[20] Rotational rheological tests are performed on proportionally optimized hydrogels, and the results suggest that different groups of hydrogels all possess shear thinning properties (Figure 3a). Many non-Newtonian biointoks demonstrate viscoelasticity and are accurately represented by the Herschel–Bulkley (H–B) model, expressed as \( \tau = \tau_0 + K \gamma^n \) where \( \tau_0 \) denotes the yield stress, \( n \) denotes the flow behavior index, and \( K \) represents the consistency index. The relationship between shear rate and shear stress for G15, G15P5, and G15P10 are fitted with the H–B model (Figure 3b), and all calculated H–B parameters are listed in Figure 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.[21]

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\(^{-1}\), 80.4% of the radius (\( R_d \)) demonstrates plug flow where the hydrogel has merely internal shear stress that does not exceed the yield stress and behaves like a solid.[22] 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 (Figure 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.[23] 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 (Figure 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 (Figure 3e). This shear thinning and self-healing can be repeated many times without sacrificing modulus.

Minimally invasive surgery 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 (Figure 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 in situ bioprinting based on the long-range extrusion procedure. In addition, the velocity of the extruded filament (\( V_{ink} \)) and the nozzle feed velocity (\( V_{max} \)) should be matched to obtain the desired printed pattern; when \( V_{ink} \) is lower than \( V_{max} \), 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 (Figure S9a,b, Supporting Information). As shown in Figure 3b, filaments with diameters of 1.5–2.2 mm hold fidelity and 3D accumulation capacity, where the corresponding ranges of \( V_{ink} \) and \( V_{max} \) 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 Fu is proposed and defined as \( Fu = L/(a+b) \) 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 \( Fu < 1 \), filaments aggregate and appear as a droplet under surface tension and go against multilayer printing, while in underfusion where \( Fu > 1 \), 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 \( Fu = 1 \) be obtained (Figure 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 (Figure 3j; Video S1, Supporting Information).

2.3. Evaluation of Robot-Assisted Bioprinting under Minimally Invasive Surgery

Minimally invasive surgery 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.\(^{[24]}\) Surgical tools must pivot around
these incisions that are called RCM constraints\textsuperscript{[25]} (Figure 4a). Due to the redundancy associated with the additional degree of freedom (DoF), the 7-axis robotic arm is capable of minimally invasive surgery at any spatial point as the RCM constraint actively, which provides greater flexibility\textsuperscript{[26]} and possesses a simple alignment procedure compared to passive RCM approaches,\textsuperscript{[27]} including the da Vinci system of Intuitive Surgical, Inc. 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 fibers are integrated (Figure 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\textsuperscript{[28]} proposed a “manipulability measure”, and Salisbury\textsuperscript{[29]} 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 incision constraint. Inspired by Stucco and Salcudean,\textsuperscript{[30]} the global isotropy index (GII) is used to provide a measure of available RCM locations. The GII can be calculated as

\[
GII = \frac{\min_{X_i,\text{workspace}} \sigma_{\min}(X_i)}{\max_{X_i,\text{workspace}} \sigma_{\max}(X_i)}
\]

where $\sigma_{\min}(X_i)$ is the smallest singular value of the Jacobian J at workspace position $X_i$, and $\sigma_{\max}(X_i)$ is the largest singular value of the Jacobian J at workspace position $X_i$. 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 root mean square (RMS) deviation is displayed in Figure 4c. The best RCM location possesses a minimal deviation of 0.177 ± 0.011 mm, and the zigzag, star and circle trajectories performed at which are listed in Figure 4e to demonstrate the kinematic accuracy on straight lines, arcs and large corners trajectory elements, which shows the coincidence between theoretical (blue lines) and actual trajectories (red lines) (Video S3, Supporting Information). The same zigzag trajectory is performed with a hand-held procedure under the minimal invasive constraint (Figure S13b, Supporting Information), and the RMS deviation reaches 0.896 ± 0.172 mm, which is fivefold larger than that of the robotic arm (Figure 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 (Figure 4f,g). In addition to the advantages in terms of accuracy and speed stability, the proposed 7-axis bioprinting robot exhibits superior flexibility compared to manual operation. Due to the similar structure with a human arm, the bioprinting robot possesses 7 DoFs and the redundancy property, further bringing with the flexibility. More prominently, however, the 7-axis manipulator allows a much wider rotation angles at each joint than a human arm. For instance, the maximum rotation angle of the elbow in human does not exceed 180°, whereas the corresponding joint range in the robot approaches 360°. The superiority of the rotation range at each joint provides the 7-axis manipulator with a wider workspace (Figure S12a, Supporting Information) and more configurations to accomplish a specific motion, which theoretically provides high flexibility of the robot manipulator. In the practical application of PROM repair, for multiple amniotic sac positions surrounding the manipulator, it is capable to solve the feasible configurations to accomplish the subaqueous bioprinting treatment while ensuring manipulability (Figure S12b,c, Supporting Information).

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,\textsuperscript{[31]} hydrogel filaments tend to slip off the substrate when the printing angle decreases. The fetal membranes 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 Figure 4h.

An in vitro amniotic sac model is fabricated in accordance with the geometry and dimensions of the human amniotic sac, and it is placed and punctured according to the calculated optimal RCM and incision location. Although theoretically optimal, the calculated incision site might not be available in practice due to the potential interference between the end-effector and the fetus within the real amniotic sac, while this issue can be addressed through alternative incision sites, or changing the position of the pregnant woman\textsuperscript{[32]} (Figure S11, Supporting Information). In this optimized scenario with great visual contrast, the 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 the minimal invasive constraints, and the printed hydrogel patch is compact with a gel-rivet structure embedded in the agarose substrate. The presence of the gel-rivets can be observed in the side view, and the remaining gel-rivets embedded in the agarose gel substrate (marked in red) can be identified after the hydrogel patch is peeled off (Figure 4i; Video S2, Supporting Information). With a focus on the robot’s global movement and the subaqueous bioprinting effects, the bioprinting process within the amniotic sac model demonstrates the feasibility and future application potential of GMPD hydrogels, the subaqueous printing process of the 7-axis bioprinting robot.

### 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,
Figure 4. Evaluation of robot-assisted bioprinting under minimally invasive surgery. 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 (marked in red). (Scale bar: 1 cm).
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 fetal membranes, indicating favorable sealing performance underwater. The same tests conducted on the intestinal mucosa of pigs presents similar results (Figure S14d, Supporting Information).

Lap shear tests are conducted according to the ASTM F2255-05 standard, and the shear strength between the

---

**Figure 5.** Sealing evaluation of subaqueous bioprinting on in vitro models. a) Schematic diagram, results, and photographs of various burst pressure test. FM: fetal membrane. 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 (I: modified syringe, II: fresh human fetal membrane, III: filleted poultry breasts). (n = 3 independent tests) (ns: p > 0.05; *: 0.01 < p < 0.05; **: 0.001 < p < 0.01; ***: p < 0.001)
hydrogel and the tissues is measured using two glass slides coated with human fetal membrane as the substrate. The shear strength of the cross-linked GMPD hydrogel reaches 33.2 ± 2.5 kPa that 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) (Figure 5b). As these commercial bio-glues do not possess the subaqueous formability, burst pressure tests and long-term sealing tests are not performed. For a comprehensive comparison, we have summarized the ex vivo experimental results that take fetal membranes as substrates (mainly burst pressure, and adhesive strength) reported in studies of PROM sealants, as shown in Table S1 (Supporting Information). The comparison on data level demonstrates the superior performance of GMPD hydrogels in terms of wet tissue adhesion. 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 (Video S4, Supporting Information). Based on the size of ruptures (2–6 mm) and the thickness of myometrium (≈12 mm) in a pregnant woman at the gestation age of 28 weeks,[31] a dense bilayer hydrogel patch measuring 15 × 15 mm possessing six rivet structures with 5 mm in length is designed and printed where the rivets are embedded in the myometrium (resembled by agarose gel in the experiments). With the ability to fabricate structures with 3D shapes, the in situ bioprinting approach prepares hydrogel patches with rivets that are embedded in the tissue and complement the interfacial adhesion of the hydrogel (Figure S19, Supporting Information).

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 sixfold in comparison to samples without rivets, from 2.43 ± 0.65 kPa to 14.53 ± 2.49 kPa (Figure 5c; Video S5, Supporting Information). The significant improvement in adhesion performance can be illustrated in the scheme of the tensile test (Figure S15, Supporting Information), where the presence of gel rivets enhanced the adhesion at the patch-substrate bonding interface in the form of mechanical forces. In the regular adhesive strength test that without gel rivets, the detachment of adhesion occurred at the weaker bonding interface, whereas the gel patch remained attached to the substrate until the substrate was fractured in the tests with gel rivets. This demonstrates the effectiveness of gel rivets in enhancing the hydrogel adhesion. Furthermore, 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 sevenfold (Figure S14a,b, and Video S6, Supporting Information).

An in vitro uterus model is established to simulate the “amniotic fluid – fetal membrane – myometrium” anatomical relationship in the uterus, where the fetal membranes are punctured to mimic PROM for quantitatively evaluating the sealing performance of various hydrogels through the fluid content inside the model. Different groups of hydrogels are printed subaquously 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. To demonstrate the instant sealing effect of our subaqueous bioprinting strategy, we removed the muscle layer from the in vitro uterus model, leaving with a thin ruptured fetal membrane wrapping underneath to accelerate the leakage of the content fluid. The subaqueous bioprinting demonstration inside the uterus model reveals the rapid sealing effect of the hydrogel patch for PROM (Video S7, Supporting Information), and the optimal G15P5 sealant maintains a leakage profile similar to that of the intact fetal membranes during the 21 day observation period, 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 intracorporal 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) (Figure S16a, Supporting Information), 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 (Figure S16c, Supporting Information). The results of the CCK-8 assay in Figure S16b (Supporting Information) also show that the cells proliferate well in the experimental groups.

Pregnant rabbits at mid-gestation are considered an approved model in the studies of the treatment for PROM sealing for their affordable housing demands, costs, and large litter sizes, and are therefore selected for intracorporal research and evaluation. The average duration of pregnancy in rabbits is 32 days, and they deliver approximately eight offspring per litter with a bicornuate uterine, which is different from that of humans. There exist overlaps between the amniotic sacs in the rabbit’s abdominal cavity, rendering the access to a specific amniotic sac in a minimally invasive approach. Thus, a laparotomy is required to expose the entire uterus and number as well as group the amniotic sacs for experiments. According to previous studies,[34,35] one to two amniotic sacs in one rabbit are selected as the experimental group and positive control group (Figure 6a). Sacs in the experimental group are punctured with a 16-gauge (1.9 mm) needle to approach real rupture sizes in human, 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. A diagram (Figure S20, Supporting Information) and practical photographs (Figure S21, Supporting Information) of the bioprinting process of the I-shape hydrogel patch have been added to the Supporting Information. At a gestational age of 31 days, the rabbits are euthanized for a second hysterotomy to evaluate the sealing performance (Figure 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
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. FM: fetal membrane. (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
dissection and opening of the gestational sac, no intraamniotic adhesions or amniotic bands are observed. Further data on the outcome are presented in Figure 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 fetal membrane, and these eight sacs remain full of amniotic fluid. The remaining two patches separated from the fetal membrane 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 ± 4.7 g compared to 8.6 ± 3.1 g in the positive control group, indicating that fetal development stops shortly after fetal membrane puncture and that fetuses are partially resorbed. Both gel sealant and fetal membrane 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 fetal membranes. At larger magnifications, the GMPD hydrogel is observed to be well integrated with fetal membrane, completing the restoration of membrane integrity and even exhibiting re-epithelialization. (Figure 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, attracts inflammatory cells, and induces collagen fibers to encapsulate them in a dense fibrous capsule.[36] Through HE staining, it is observed that the implanted hydrogel in first week exhibits an acute foreign body reaction that is consistent with expectations,[37] with infiltration at the hydrogel borders by mononuclear cells, as evidenced by their nuclear and cellular morphology. With the increase of implantation period, the population of inflammatory cells around the hydrogel decreases significantly, indicating a reduced immune response. Finally, after 3 weeks of implantation, the inflammatory cells are barely discernible adjacent to the hydrogel, suggesting the immune response evident in 1 week has basically disappeared (Figure S17a, Supporting Information). This tendency for the immunoinflammatory response to diminish rapidly over time is indicative of the lower immunogenicity of the GMPD hydrogels.[38] The morphology and thickness of the fibrous capsule can be considered metrics of immunogenicity. It is apparent through Masson’s trichrome staining that the GMPD hydrogels present low immunogenicity and favorable biocompatibility that can be considered an ideal biomaterial for intracorporal applications.

3. Discussion

In situ bioprinting has been proven to be a potential approach for the treatment of tissue injuries due to its immediacy, convenience and functionality,[42] but challenges remain when applied to applications of internal tissue repair. For example, it is difficult to ensure a rapid and stable crosslinking of bioink in a wet and possible bleeding environment around the defect, and it takes effort to achieve high precision treatment for complicated intracorporal tissues through a minimally invasive approach. The inside of amniotic sacs can be considered as the most challenging location to attempt in situ 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 situ bioprinting inside the liquid surroundings through our robot-assisted minimally invasive approach not only extends the applicable scenarios of in situ bioprinting, but also provides a strategy for the treatment of internal tissue injuries including PROM.

To date, a large number of PROM treatments have been attempted, including mixtures of maternal platelets and fibrin cryoprecipitate,[43] collagen/gelatin plugs,[44] synthetic polymer sealants,[45] and laser welding approaches,[46] these have met with preliminary success, but no clear pathway to a clinically viable solution has emerged after decades of research. As Winkler et al. contended,[47] an ideal membrane sealing material would have mechanical properties similar to those of fetal membranes, subaqueous formability, and non-immunogenicity, 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 Yul[13] and mussel glue by Fan et al.[12] Even so, creating 3D structures from biomaterials is considered more significant, not only for stronger sealing but also to match defective tissues for subsequent tissue repair and regeneration. Furthermore, the creation of 3D structures in liquid environment can be considered as an extension of the bath-assisted bioprinting. Broadly speaking, gel baths such as the FRESH technique[48] and oil bath-based printing methods[49,50] 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 gelation due to the dilution of the gel precursors. In cooperation with the in situ cross-linking process, the GMPD hydrogel ensures ultrafast gelation and stable printability in liquid environment, which provides a versatile paradigm for multiple tissues repair through in situ bioprinting.
The biocompatibility of materials is essential for their in vivo applications; hence, at the beginning of the development of bioinks, we chose GelMA and PEGDA, which have been commonly used in tissue engineering, as the components to ensure the controllable biocompatibility of the GMPD hydrogels. Previous studies on GelMA have indicated that it boasts a variety of bioactive motifs, including arginine–glycine–aspartic acid and matrix metalloproteinase that benefit for cell migration and proliferation.\textsuperscript{51} In addition, it presents significantly lower immunogenicity due to a lower number aromatic group.\textsuperscript{52} In research containing GelMA hydrogels applied in animal experiments, scholars have demonstrated that this hydrogel exhibits favorable functionality in subcutaneous implantation,\textsuperscript{53} lung sealing,\textsuperscript{54} and cartilage repair\textsuperscript{55} without eliciting noticeable immune reactions. Furthermore, GelMA hydrogels have attracted widespread attention in the construction of drug screening models, stem cell expansion and organ-on-a-chip studies, all of which have revealed the non-cytotoxicity and ability for support for the physiological expression of cells.\textsuperscript{56} PEGDA, on the other hand, is a derivative of PEG, and presents intrinsical biocompatibility.\textsuperscript{57} It is noteworthy that PEGDA hydrogels with molecular weights above 400 Da are non-toxic, non-immunogenic, readily cleared by the kidneys and is approved by FDA for various clinical uses.\textsuperscript{58} The PEGDA hydrogels used in our study possess a molecular weight of 1 kDa and thus also appropriate for intracorporal applications.

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),\textsuperscript{59} while merely robot-assisted fetal surgeries and postoperative recovery. A recent review reported with our study results, and would affect the success rate of fetal surgery 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.\textsuperscript{20,31,62} In addition to fulfill 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.\textsuperscript{63,64} 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.\textsuperscript{65} Despite these comprehensive advantages demonstrated by the 7-axis bioprinting robot over manual operations and other mechanisms, it is inevitable that the intelligence of the robot is far from human beings. Consequently, when faced with dynamic, vulnerable tissues, the collisions with the defect tissues may occur due to the mismatch between the preset path of manipulator and the varying microenvironment. For the implementation of robot-assisted subaqueous bioprinting in the living, advances in robotics are needed, including enhancing the external perception and intelligence. For instance, developing machine vision to track the real-time movement and deformation of the amniotic sacs, controlling the contact force between the printhead and the tissues through the joint torque sensors, and utilizing artificial intelligence to handle and integrate these external messages to constitute a closed-loop bioprinting. Statistically, most cases of PROM occur at a gestational age of 25–32 weeks,\textsuperscript{66} and ≈60% of premature deliveries occur within the first week after PROM;\textsuperscript{67} dyson-togentic 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 ≈30%.\textsuperscript{68} As long as the gestational age 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 in vitro uterus model to 21 days. Although the intact fetal membrane 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 fetal membrane. 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 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 fetal membrane are much different from those in human or nonhuman primates, which prevents the robot-assisted minimally invasive operation and hydrogel rivets from being embedded. Due to the shorter gestation of rabbits, only 9 days of intracorporal sealing are performed. Nevertheless, the in situ bioprinting approach still reveals the sealing integrity of fetal membrane and promising re-epithelialization. In subsequent studies, the minimally invasive bioprinting process could be gradually implemented in larger animals, such as sheep or primate models with an approximate gestation of 160 days and similar physiology to humans, eventually making minimally invasive in situ bioprinting possible for the treatment of PROM in humans.
As a typical medico-engineering collaborative study, future studies are required to investigate many aspects, including: i) a photo-crosslinking approach with long wavelengths to avoid unknown impacts on the fetal development; ii) robust controller design for further application of active robot-assisted minimally invasive operations; 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 in situ bioprinting approach for tissue sealing offers a promising option for blocking leakage of amniotic fluid, prolonging the gestational age 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. Experimental Section

Preparation of GMPD Hydrogel: Methacrylate gelatin (GelMA), poly (ethylene glycol) diacylates (PEGDA), and lithium phenyl-2,4,6-trimethylbenzylphosphinate (LAP) were purchased from Engineering for life (EFL Inc., China). And the two gel precursors were dissolved in phosphate 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 to form GMPD hydrogel, and predict the shear stress and velocity distribution during extrusion-based bioprinting. The derivation of computation model was used to simulate the rheological behavior of GMPD hydrogel and predict the shear stress and velocity distribution. 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 was obtained by comparing with the theoretical trajectory.

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 Supporting Information. Briefly, for laminar, isothermal flow of an incompressible fluid with no-slip boundary conditions, the shear stress of the inner wall of needle τwall can be calculated from Equation 3, i.e.,

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

where Q stands for the flow rate of extrusion, τ0 denotes the yield stress, R is the inner radius of needle, n is the flow index, and K is the flow consistency index.

Once τwall was determined, the shear rate γ and velocity u profiles could be calculated for all positions within the flow from Equations 4 and 5, i.e.,

\[ \gamma = \left( \frac{\tau_{wall}}{K} \right) \left( \frac{1}{R} - \frac{\tau_0}{\tau_{wall}} \right) \left( R^2 - 1 \right) R_0 \leq R \leq R \]

\[ u = \left( R^2 - 1 \right) \left( \frac{\tau_{wall}}{K} \right) \left( 1 - \frac{\tau_0}{\tau_{wall}} \right)^{\frac{n+1}{n}} - \left( \frac{\tau_0}{R} \right)^{\frac{n+1}{n}} \left( R^2 - 1 \right), R_0 \leq R \leq R \]

where R0 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. 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 was 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 G15PS hydrogel precursor was injected and in situ cross-linked 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 °C. Once the incision was created on the FM, fluid contained leaks through the puncture site, and 500 μL G15PS hydrogel precursor was immediately printed subaqueously to
seal the leakage. Burst pressure peaks were detected by increasing the hydraulic pressure above, and it reflected 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.

**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 fetal membrane 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 fetal membrane and attached to glass slides with cyanoacrylate glue. Then, 200 µL freshly collected human fetal membrane was cut into 2.5 cm squares and was divided by the bonding area to find the adhesive strength.

**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 amniotic vesicles (normally 20 mL) and fetal membrane was wrapped underneath and the poultry breast was secured on the outside to simulate the anatomical structure of fetal membrane 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 fetal membrane 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. The fetal membranes were collected with the informed written consent of all participants according to the protocol approved by the Ethics Committee of Foshan Maternal and Child Health Hospital.

**Rat’s Subcutaneous Implantation:** Six male 12-week-old Sprague Dawley rats weighing ~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. G15PS hydrogel precursor was injected into the subcutaneous pockets and in situ cross-linked 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).

**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⁻¹ (Macklin, Shanghai, China) and xylazine 6 mg kg⁻¹ (Macklin, Shanghai, China), followed by anesthesia with isoflurane (1–1.5%) in oxygen at 1.5 L min⁻¹ (RWD Life Science, Shenzhen, China). Preoperative medroxyprogesterone acetate 9 mg kg⁻¹ (Rawhan, Shanghai, China) was administered intramuscularly for tocolysis, and Penicillin G 30000 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 G15PS gel patch with I-shape was printed at the defect to seal the puncture site (n = 11). As shown in Figure S20 (Supporting Information), the printhead of the end-effector was inserted into the amniotic sac along the rupture site on the fetal membrane, then the hydrogel was injected and gelled in situ under excitation light. At this moment, the end-effector was rotated around the normal direction of the rupture site, and a thin hydrogel sheet was formed in the inner side of the defect, which was the lower half of the I-shape hydrogel patch. Next, the printhead slowly withdrew from the rupture site, and the upper half of the I-shape gel patch was constructed by continuing the injection, in situ bioprinting and slight rotation on the outside of the defect of the fetal membrane. Finally, the I-shape hydrogel was constructed an integrated form to seal the rupture site with the upper and lower surface of the I-shape, and this structure ensured that the patch be embedded in the defect. 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 31st day of gestation, rabbits were anesthetized with isoflurane (1–1.5%) in oxygen at 1.5 L min⁻¹ 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-gauge 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).

**Histology and Immunofluorescence:** Histology and immunofluorescence studies were performed according to previous studies. Briefly, the tissue samples from rat subcutaneous implantation and rabbit fetal membrane sealing were fixed in 4% paraformaldehyde overnight at 4 °C. After fixation, 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 tissue engineering application of this study: the treatment for PROM is brought up during Foshan Maternal and Child Health Hospital during this study. The data that support the findings of this study are available from the author.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The authors appreciate the consultation and guidance from clinical perspective provided by Dr. Zhengping Liu and Dr. Dongxin Lin from Foshan Maternal and Child Health Hospital during this study. The application of this study: the treatment for PROM is brought up during the communication with these obstetricians and the advancement of this work incorporates clinical considerations including surgical procedures, which might provide the study with a promising clinical translational potential. The authors appreciate Dr. Lu Feng for her critical reading and linguistic embellishment to this study. This study was supported in part by the National Nature Science Foundation of China under Grants 51922059 and 52075285, in a part by the Beijing Natural Science Foundation under Grant JQ19010.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords
7-axis bioprinting robots, fetal membrane repairs, in situ bioprinting, tissue engineering

Received: July 1, 2022
Revised: August 18, 2022
Published online: October 3, 2022

[1] R. L. Goldenberg, J. F. Culhane, J. D. Iams, R. Romero, Lancet 2008, 377, 75.
[2] J. D. Iams, R. Romero, J. F. Culhane, R. L. Goldenberg, Lancet 2008, 377, 164.

[3] H. Niknejad, H. Peirovi, M. Jorjani, A. Ahmadiani, J. Ghanavi, A. M. Seifalian, Eur. Cells Mater. 2008, 15, 83.
[4] Y. R. Devaud, S. Züger, R. Zimmermann, M. Ehrbar, N. Ochsenbein-Küble, Fetal Diagn. Ther. 2019, 45, 102.
[5] R. Papanna, L. K. Mann, S. C. Tseng, R. J. Stewart, S. S. Kaur, M. M. Swindle, T. R. Kyriakides, N. Tatevian, K. J. Moise Jr, Placenta 2015, 36, 888.
[6] V. Pensabene, P. P. Patel, P. Williams, T. L. Cooper, K. C. Kirkbride, T. D. Giorgio, N. B. Tulipan, Ann. Biomed. Eng. 2015, 43, 1978.
[7] C. Haller, W. Buerzle, A. Kivelio, M. Perrini, C. Brubaker, R. Gubeli, A. Mallik, W. Weber, Acta. Biomater. 2012, 8, 4365.
[8] W. Zhao, T. Xu, Biofabrication 2020, 12, 045020.
[9] M. Albanna, K. W. Binder, S. V. Murphy, J. Kim, S. A. Qasem, W. Zhao, J. Tan, I. B. El-Amin, D. D. Dice, J. Marco, et al., Sci. Rep. 2019, 9, 1856.
[10] K. Ma, T. Zhao, L. Yang, P. Wang, J. Jin, H. Teng, D. Xia, L. Zhu, L. Li, Q. Jiang, et al., J. Adv. Res. 2020, 23, 123.
[11] A. Uriculo, I. Poli, L. Brandolino, P. Raffa, V. Scattolini, C. Laterza, G. G. Gieöe, E. Zamaiti, C. Selmin, M. Magnussen, Nut. Biomed. Eng. 2020, 4, 901.
[12] C. Fan, J. Fu, W. Zhu, D.-A Wang, Acta. Biomater. 2016, 33, 51.
[13] H. Yuk, J. Wu, T. L. Sarrafian, X. Mao, C. E. Varela, E. T. Roche, L. G. Griffiths, C. S. Nabzydk, X. Zhao, et al., Nat. Biomed. Eng. 2021, 5, 1131.
[14] Y. Chen, J. Zhang, X. Liu, S. Wang, J. Tao, Y. Huang, W. Wu, Y. Li, K. Zhou, X. Wei, Sci. Adv. 2020, 6, eaba7406.
[15] C. Zhou, Y. Yang, J. Wang, Q. Wu, Z. Gu, Y. Zhou, X. Liu, Y. Yang, H. Tang, Q. Ling, et al., Nat. Commun. 2021, 12, 5072.
[16] H. Mogami, A. H. Kishore, Y. Akgül, R A. Word, Sci. Rep. 2017, 7, 13139.
[17] E. Avilla-Royo, N. Ochsenbein-Külble, L. Vonzun, M. Ehrbar, Biomater. Sci. 2022, 3695.
[18] R. Nagyné Elek, T. Haidegger, Acta. Polytech. Hung. 2019, 16, 141.
[19] W. Schuurman, P. A. Levett, M. W. Pot, P. R. van Weeren, W. J. Dhetr, D. W. Hutmacher, F. P. Melchels, T. J. Klein, J. Malda, et al., Macromol. Biol. 2013, 13, 551.
[20] D. Chimene, R. Kaunas, A. K. Gharawar, Adv. Mater. 2020, 32, 1902026.
[21] N. Paxton, W. Smolan, T. Böck, F. Melchels, J. Groll, T. Jungst, et al., Biofabrication 2017, 9, 044107.
[22] F. P. Melchels, M. M. Blokzijl, R. Levato, Q. C. Peiffer, M. De Ruijter, W. E. Hennink, T. Vermondon, J. Malda, Biofabrication 2016, 8, 035004.
[23] Y. He, F. Yang, H. Zhao, Q. Gao, B. Xia, J. Fu, et al., Sci. Rep. 2016, 6, 29977.
[24] V. Vitiello, S.-L. Lee, T. P. Cundy, C.-Z. Yang, IEEE Rev. Biomed. Eng. 2012, 6, 111.
[25] H. Sadeghian, F. Zokaei, S. H. Jazi, J. Intell. Rob. Syst. 2019, 95, 901.
[26] H. Su, S. Li, J. Manivannan, L. Bascetta, G. Ferrigno, E. De Momi, 2019 Int. Conference on Robotics and Automation (ICRA), IEEE, Piscataway, 1323–1328.
[27] M. T. Getman, M. L. Blute, C. K. Chow, R. Neururer, G. Bartsch, R. Peschel, Urology 2004, 64, 914.
[28] T. Yoshikawa, Int. J. Rob. Res. 1985, 4, 3.
[29] J. K. Salisbury, J. J. Craig, Int. J. Rob. Res. 1982, 1, 4.
[30] L. Stocco, S. E. Salcudean, F. Sassani, Tec. Autom. 1998, 16, 595.
[31] W. Zhao, H. Chen, Y. Zhang, D. Zhou, L. Liang, B. Liu, T. Xu, et al., Bioeng. Transl. Med. 2022, e10303.
[32] M. A. Haogland, D. Chatterjee, Paediatr. Anaesth. 2017, 27, 346.
[33] A. B. Caughey, J. N. Robinson, E. R Norwitz, Rev. Obstet. Gynecol. 2008, 1, 11.
[34] E. Gratacos, J. Wu, N. Yesildaglar, R. Devlieger, R. Pijnenborg, J. A. Deprest, et al., Am. J. Obstet. Gynecol. 2000, 182, 142.
[35] N. Ochsenbein-Küble, J. Jani, L. Lewi, G. Verbist, L. Vercruysse, B. Portmann-Lanz, K. Marquardt, R. Zimmermann, J. Deprest, et al., Am. J. Obstet. Gynecol. 2007, 196, 263.
