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Research Letter

Development of a Prototype Tissue-Specific Photopolymerizable Bioengineered Matrix Utilizing Temperature-Responsive Copolymeric Hydrogels for Multi-Material Volumetric Photopolymerization

Alexander Yu. Pulver[1*], Irina V. Ostankova[2], Asiyat A. Magomedrasulova[3], Julia Ol. Potapova[4], Ekaterina A. Vorontsova[5], Soltan Kh. Soltanov[6], Natalie A. Pulver[7]

[1]Laboratory of Postgenomic Research, Research Institute of Experimental Biology and Medicine, N.N. Burdenko Voronezh State Medical University, 10, Studentskaya Str., Voronezh, 394036, Russia
[2]Department of High-Molecular Compounds and Colloid Chemistry, Voronezh State University, 1, Universitetskaya Pl., Voronezh, 394018, Russia
[3]Department of Operative Surgery with Topographic Anatomy, N.N. Burdenko Voronezh State Medical University, 10 Studencheskaya Street, Voronezh, 394036, Russia
[4]Moscow City Spinal Neurosurgical Center, L.A. Vorokhobov City Clinical Hospital No. 67, 2/44 Salama Adil Street, Moscow, 123423, Russia
[5]Voronezh City Polyclinic No. 10, 82, 20th Anniversary of October Street, Voronezh, 394006, Russia
[6]Federal State Budgetary Institution "V.A. Almazov National Medical Research Center" of the Ministry of Health of the Russian Federation, 2, Akkuratova Str., St. Petersburg, 197341, Russia
[7]Department of Health Management, N.N. Burdenko Voronezh State Medical University, 10, Studentskaya Str., Voronezh, 394036, Russia
E-mail: pulver.ibs@gmail.com

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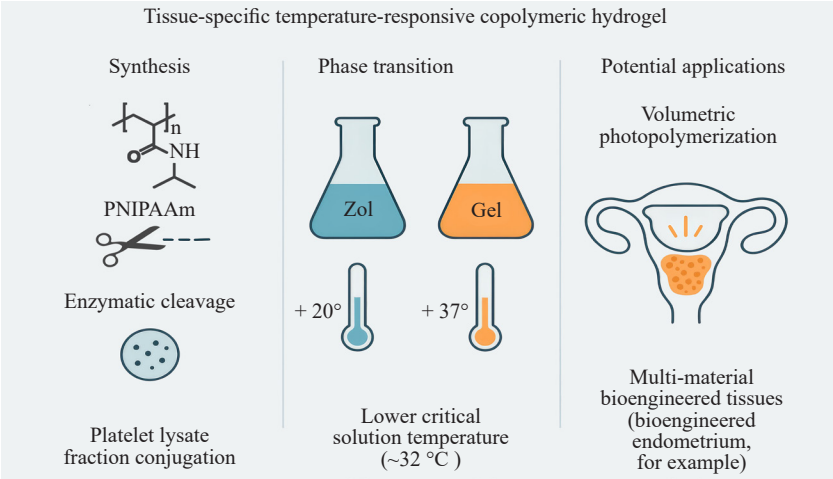

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Abstract: This study addresses key limitations in tissue engineering—specifically, the inability of traditional additive bioprinting methods to fabricate complex, multi-material structures with the resolution and speed required for functional tissues. Volumetric photopolymerization methods, such as xolography and pulsed holographic photopolymerization, offer superior resolution and simultaneous whole-volume fabrication but require advanced materials. We present a prototype "liquid bioengineered tissue" matrix designed for such volumetric methods, specifically targeting bioengineered endometrium. The innovation is a biocompatible, temperature-responsive, photopolymerizable hydrogel based on Poly(N-Isopropylacrylamide) (PNIPAAm), with a Lower Critical Solution Temperature (LCST) near body temperature. This enables a phase transition from a homogeneous solution at room temperature to a precipitated state under physiological conditions. The matrix is made tissue-specific by conjugating solubilized glycoproteins and glycosaminoglycans from endometrial Extracellular Matrix (ECM) into the hydrogel. To enhance biological activity, a glycoprotein fraction from platelet lysate is chemically incorporated, extending growth factor stability. Synthesis involved radical polymerization of N-isopropylacrylamide to form PNIPAAm, enzymatic digestion of endometrial ECM, and subsequent component conjugation. The resulting hydrogels were purified and characterized. Particle size analysis confirmed nanoparticle formation suitable for a "morphogenetic matrix". The hydrogels remained optically transparent below the LCST, which is critical for volumetric photopolymerization. This PNIPAAm-based matrix, incorporating tissue-specific ECM components and platelet lysate factors, represents a foundational step towards enabling high-resolution volumetric photopolymerization for complex tissue engineering. While realizing pulsed holographic photopolymerization remains challenging, this matrix provides essential compatible materials for future volumetric bioprinting advances.

***Keywords*:** bioengineered matrix, Extracellular Matrix (ECM), platelet lysate, Poly(N-Isopropylacrylamide) (PNIPAAm), pulsed holographic photopolymerization, temperature-responsive hydrogels, volumetric photopolymerization, xolography

Abbreviations

ECM	Extracellular Matrix (structural biological network of tissue proteins/glycosaminoglycans)
IR	Infrared Spectroscopy (analytical technique for molecular characterization)
KPS	Potassium Persulfate (chemical initiator for radical polymerization)
LCST	Lower Critical Solution Temperature (phase transition point of thermoresponsive polymers)
LBT	Liquid Bioengineered Tissues (fluid matrices for volumetric photopolymerization)
PBS	Phosphate-Buffered Saline (isotonic pH-stabilizing solution)
PNIPAAm	Poly(N-Isopropylacrylamide) (thermoresponsive polymer with LCST ~ 32 °C)
PVCL	Poly-N-Vinylcaprolactam (alternative thermoresponsive polymer to PNIPAAm)
ROS	Reactive Oxygen Species (toxic radicals generated during photopolymerization)
Xolography	Volumetric 3D printing method using dual-wavelength photoinitiation

1. Introduction

Tissue engineering aims to create functional biological tissues by combining cells, biomaterials, and biochemical factors. A key challenge is the fabrication of complex multi-material structures that can replicate the intricate architecture of natural tissues, including capillary networks with diameters as small as 5-10 μm.[1] Traditional additive bioprinting methods, such as extrusion and inkjet printing, are limited in their ability to achieve the resolution required for medical applications.[1-3] These limitations have driven the exploration of alternative approaches, among which volumetric photopolymerization methods stand out for their potential to offer higher resolution and faster fabrication times.

We focus on methods that, with further development, could be suitable for "medical-grade bioprinting," such as xolography[4] and a recently proposed, largely theoretical, pulsed holographic photopolymerization technique.[1] The latter, in particular, has the potential to achieve submicron resolution and multi-material capabilities, addressing key limitations of existing methods. Additionally, we present the first practical step toward realizing this approach: the

development of a prototype bioengineered endometrium using temperature-responsive copolymeric hydrogels.

2. Beyond the basics: why additive bioprinting demands a paradigm shift

Traditional additive bioprinting technologies are inadequate for creating fully functional tissues due to several limitations. Extrusion printing achieves resolutions of only 150-200 μm, while inkjet printing, despite a theoretical capability of 20 μm, is constrained by the availability of biocompatible materials.[1-2] The layer-by-layer fabrication process is slow, with production rates of 1-20 mm^3/h, rendering the printing of highly detailed organs too time-consuming for cell survival and impractical for laboratory-scale experiments. Photopolymerization, often employed to enhance precision, generates toxic Reactive Oxygen Species (ROS) that trigger apoptosis. Additionally, integrating multiple materials requires complex post-processing, further reducing efficiency.[1,3] These factors highlight the urgent need for innovative technologies capable of replicating complex cytoarchitectures, including vascular networks.

3. Volumetric methods: a brief overview

Volumetric photopolymerization methods create structures simultaneously throughout the entire volume, overcoming the challenges of layer-by-layer printing.[1] Notable examples include:

• Holographic structuring photopolymerization, which produces objects in 1-10 seconds with resolutions in the tens of micrometers;[5]

• Computed axial lithography, offering fabrication times in seconds with resolutions around 300 μm;[6]

• Xolography,[4,7] where recent scientific literature reports resolutions of approximately 20 μm for positive features and 125 μm for negative features, while industry reviews and official materials from the developer indicate that commercial systems can achieve resolutions as fine as 5 μm using dual-color photoinitiators.

These methods significantly enhance speed and precision. However, submicron resolution (0.1-0.2 μm) and robust multi-material capabilities (present only in a limited form in xolography[7-8]) remain elusive. To tackle these challenges, we have theoretically developed the pulsed holographic photopolymerization method, based on a mathematical three-dimensional model, targeting resolutions of 0.1-0.2 μm. This approach utilizes a pre-polymerization, optically transparent "morphogenetic matrix" composed of the materials discussed herein, enabling the creation of multi-material bioengineered structures at near-natural scales. The photopolymerization occurs instantaneously, with all components formed simultaneously. The device designed for this purpose has been named the Holographator.[1]

4. Selection of "Bioinks"

Since bioengineered tissues are intended to support specific cell types and form functional tissues, the materials used must be not only biocompatible but also appropriately tissue-specific.[1,9] For the fabrication of multi-material bioengineered tissues via volumetric methods (see "*pre-polymerization morphogenetic matrix*"[1]), tissue-specific temperature-responsive copolymeric hydrogels with well-controlled phase transition temperatures are indispensable. For simplicity, we refer to these as Liquid Bioengineered Tissues (LBT).

5. Designing the next generation of bioengineered tissues[1]

We plan to utilize biocompatible, photopolymerizable, temperature-responsive hydrogels with adjustable phase transition temperatures. These hydrogels will incorporate covalently bound, solubilized, functionally important glycoproteins and glycosaminoglycans from the Extracellular Matrix (ECM) of the target biological tissue. Based on Poly(N-Isopropylacrylamide) (PNIPAAm) or Poly-N-Vinylcaprolactam (PVCL), these materials exhibit a phase transition in aqueous environments at temperatures close to that of the human body (hereafter exemplified by PNIPAAm, with a Lower Critical Solution Temperature (LCST) of ~ 32 °C). Aqueous solutions of linear PNIPAAm

polymers are homogeneous at room temperature but undergo a phase transition above the LCST. This mechanism is driven by hydration-dehydration dynamics. At room temperature, water molecules are bound to PNIPAAm's amide groups via hydrogen bonds, maintaining the hydrated polymer chains in solution. As the temperature rises, thermal fluctuations weaken these bonds, causing water molecules to migrate from the polymer, reducing chain hydration. The hydrophobic nature of the polymer chains then dominates, leading to polymer precipitation. When small amounts of covalent conjugates are used, these polymer dispersions remain optically transparent before gelation, making them suitable as a base for the morphogenetic matrix in multi-material volumetric photopolymerization.

LBT must be temperature-responsive, biocompatible, and "tissue-specific," mimicking the composition of the target tissue's ECM. This is achieved by chemically conjugating functionally important ECM components (primarily glycoprotein and mucopolysaccharide fractions) during hydrogel formation, using solubilized ("liquefied") native ECM. Growth factors are also essential for cell growth and organization within the conjugated hydrogel. To this end, we plan to chemically integrate a glycoprotein fraction from platelet lysate, rich in growth and trophic factors, into the hydrogel. Platelet lysate is widely recognized for supporting cell growth in culture more effectively than traditional fetal sera. However, free growth factors degrade within 1-3 days. By chemically conjugating them into the hydrogel, their functional lifespan is significantly extended. This "platelet domain for enhanced biological activity" could also be used independently as an additive for serum-free cell cultures. Additionally, for volumetric photopolymerization, LBT must be photopolymerizable and capable of irreversibly gelling under light exposure.

6. Development of a prototype

The creation of tissue-specific, temperature-responsive bioengineered tissues involved several stages.

6.1 *Synthesis of poly(N-isopropylacrylamide) (PNIPAAm)*

Radical polymerization of N-isopropylacrylamide was conducted in distilled water using Potassium Persulfate (KPS) as the initiator and sodium metabisulfite as the co-initiator.[10] N-isopropylacrylamide was dissolved in distilled water, and the solution was degassed in an ultrasonic bath for 2-3 minutes. KPS and sodium metabisulfite were added at varying concentrations, and the reaction mixture was purged with argon for several minutes to remove residual oxygen. Polymerization was carried out at 20 °C for 8 hours with vigorous stirring (350-400 rpm) using an overhead stirrer.[11] After synthesis, the polymer solution was dialyzed against water for 7 days using a Spectra/Por dialysis membrane (molecular weight cut-off: 3,000-5,000 g/mol). The solution was then frozen and lyophilized at -50 °C to remove water.

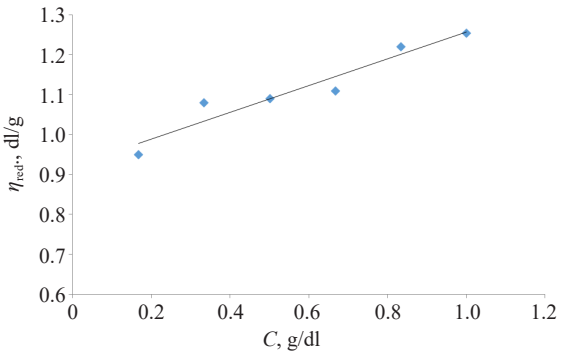

Figure 1. Dependence of the reduced viscosity of PNIPAAm aqueous solutions on polymer concentration

The molecular weight of the synthesized PNIPAAm was determined using viscometry with an Ubbelohde viscometer (d = 0.52 mm). The intrinsic viscosity (η) was estimated by extrapolating the reduced viscosity to zero polymer concentration (Figure 1).

Molecular weight was calculated using *the Mark-Kuhn-Houwink equation*:

$[\eta] = K \cdot M^{\alpha}$, where K and α are constants for the polymer-solvent system at a given temperature.

The molecular weight of the PNIPAAm samples was found to be 250,000 g/mol.

Synthesized polymer chemical structure was confirmed by Fourier-transform Infrared (IR) spectroscopy spectroscopy (Figure 2). The obtained spectrum exhibits all the characteristic absorption bands of PNIPAAm. The presence of the secondary amide group is confirmed by the N-H stretching band at approximately 3,300 cm^{-1}, the intense Amide I band (C=O stretch) at 1,645 cm^{-1}, and the Amide II band (N-H bending coupled with C-N stretching) at 1,535 cm^{-1}. A distinctive doublet at 1,385 and 1,365 cm^{-1}, corresponding to the symmetric bending of the geminal dimethyl group ($-CH(CH_3)_2$), provides clear evidence for the presence of the N-isopropyl moiety.[12]

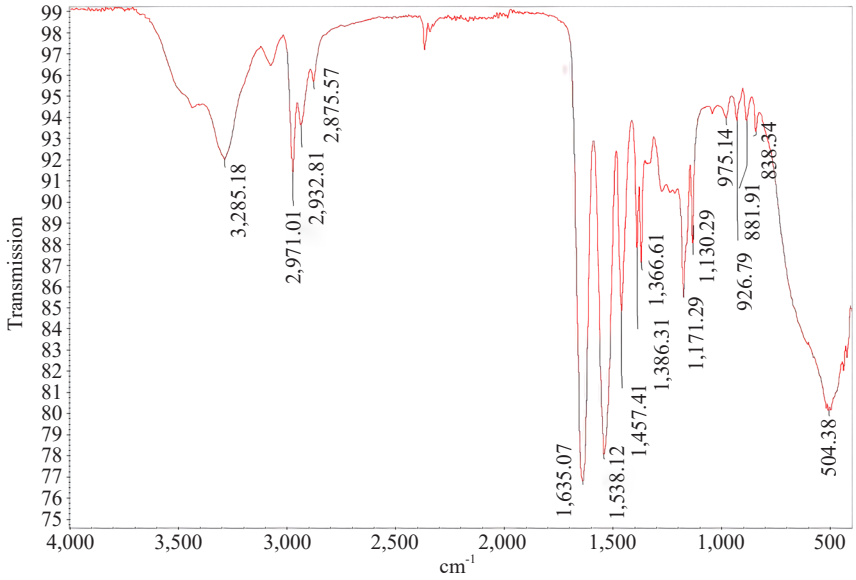

Figure 2. IR spectrum of PNIPAAm

A detailed analysis of the Amide I region reveals its complex nature. As demonstrated in studies of tacticity-controlled PNIPAAm, the band at approximately 1,645 cm^{-1} can be deconvoluted into components assigned to free, weakly hydrogen-bonded, and strongly hydrogen-bonded carbonyl groups, respectively.[13] This reflects the specific molecular configuration and hydrogen-bonding interactions within the polymer. The collective presence of these bands unequivocally confirms the successful PNIPAAm synthesis.

The Ultraviolet (UV) spectroscopy data for the polymer sample showed an absorption maximum at 219 nm in the range of 200 to 240 nm (Figure 3).

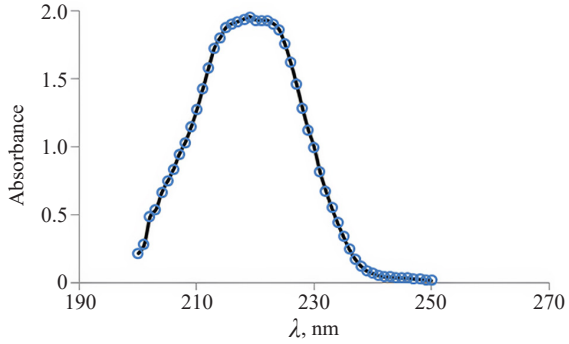

Figure 3. UV spectrum of PNIPAAm

7. Synthesis of ECM hydrogels

To prepare ECM hydrogels,[14] extracellular matrix derived from the endometrium was enzymatically digested in a solution of 1 mg/mL porcine pepsin in 0.01 $molL^{-1}$ HCl for 48 hours at room temperature with continuous stirring on a magnetic stirrer. Gelation was induced by neutralizing the digest with one-tenth the volume of 0.1 $molL^{-1}$ NaOH and one-ninth the volume of 10 × PBS. The mixture was heated to 37 °C for 1 hour with vigorous stirring. The base concentration of the ECM hydrogel was 30 mg/mL.

8. Preparation of conjugated hydrogels based on poly(N-isopropylacrylamide), extracellular matrix, and platelet lysate

PNIPAAm, ECM, and platelet lysate[15] were added in varying ratios to a 0.05-0.2% solution of glutaraldehyde in 0.1 M phosphate buffer.[16]

The reaction mixture was purged with argon for 3-5 minutes to remove residual oxygen and placed on a heated magnetic stirrer. The system was stirred vigorously at 37 ± 1 °C for 8 hours at 500-600 rpm.

After synthesis, the solutions were dialyzed against water for 7 days using a Spectra/Por dialysis membrane (molecular weight cut-off: 3,000-5,000 g/mol). The products were then frozen and lyophilized at -50 °C to remove water. For further use, water or PBS was added to the hydrogels to achieve the desired concentration, and the mixtures were sonicated at 4 ± 2 °C in 30-second cycles.

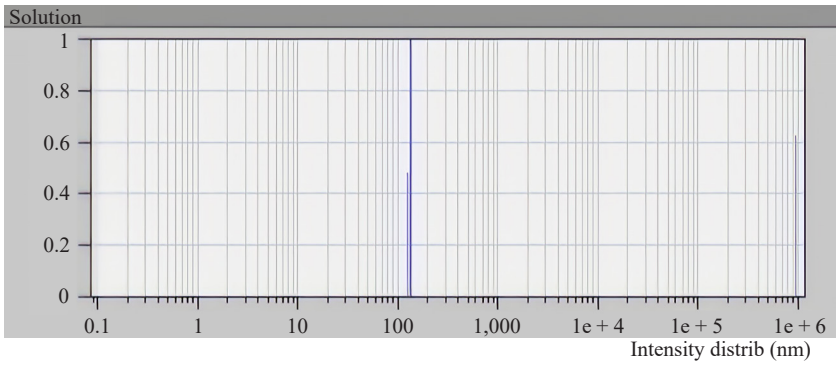

Figure 4. Particle size measurements of the synthesized hydrogels after sonication

Particle size measurements of the synthesized hydrogels after sonication were performed using a *Photocor-Complex modular dynamic and static light scattering spectrometer (Photocor, Russia)*, equipped with a 25 mW He-Ne laser ($\lambda \approx 633$ nm). Calculations were carried out using DynaLS software. The hydrodynamic radius of the particles was determined at 20 °C and a 90° angle for solutions with a concentration of 10 g/L (Figure 4). The intensity-weighted size distribution revealed a primary, sharp peak centered at approximately 250 nm, indicating the formation of a dominant population of nanoscale hydrogel particles. The distribution shows a minor but significant population of particles in the micron-scale range, with a distinct peak around 3,000 nm (3 μm). The presence of these larger particles suggests that a fraction of the hydrogel material formed aggregates or larger structures alongside the primary nanoparticles.

At concentrations above 10 g/L, the hydrogels exhibited a faint light brown color, particularly in samples containing 2% or more platelet lysate relative to PNIPAAm. Samples with lower platelet lysate content remained colorless.

9. Conclusion

In this study, we developed biocompatible, temperature-responsive PNIPAAm-based hydrogels incorporating ECM-derived glycoproteins and glycosaminoglycans, along with platelet lysate fractions for enhanced biological activity. These matrices exhibit phase transitions near body temperature, remaining optically transparent below the LCST, making them suitable for multi-material volumetric photopolymerization in tissue engineering.[17] Our prototype advances reproductive medicine by enabling high-resolution fabrication of bioengineered endometrium, potentially aiding treatments for infertility and uterine disorders, and even artificial wombs. Unlike traditional layer-by-layer bioprinting, volumetric methods like xolography and pulsed holographic photopolymerization offer superior speed and precision. Though hurdles persist, including funding for realizing pulsed holographic techniques, this work provides foundational materials for future innovations in functional tissue creation and biomedical research.

Ethical declarations

This study is theoretical and does not involve human or animal subjects, therefore, no ethical approval was required.

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No external funding was received for this research.

Conflict of interest

The authors declare that they have no competing interests.

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