



OPEN Predicted peptide scaffolds for drug screening in endometrial cancer organoids

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AlphaFold, a deep learning–based platform widely used to predict protein and peptide structures, was employed in this study to model the self-assembling peptide RFC, which demonstrated a stable α -helical structure with high confidence. This structural prediction was supported by experimental analyses, which revealed the peptide's ability to form dense fibrillar networks and robust hydrogels, particularly at higher concentrations. These hydrogels effectively supported the 3D culture of endometrial cancer organoids, which retained key tumor characteristics, including high proliferative activity and resistance to platinum-based drugs. Among tested therapeutics, Doxorubicin showed the strongest efficacy, significantly reducing organoid viability. This study highlights the predictive power of AlphaFold in elucidating peptide structures and guiding biomaterial development. The RFC hydrogel, combined with organoid modeling, represents a promising platform for advancing cancer research and precision medicine. These findings demonstrate the synergistic value of computational tools like AlphaFold and experimental approaches in creating innovative solutions for challenging biomedical applications.

Keyword AlphaFold; Self-assembling peptide; Endometrial cancer organoids

AlphaFold, developed by DeepMind, represents a groundbreaking advancement in the field of computational biology. By utilizing deep learning techniques, AlphaFold can predict protein structures with remarkable accuracy, significantly surpassing traditional methods^{1,2}. The ability to predict protein folding accurately is crucial for understanding the protein's function, interactions, and its role in various biological processes^{3–5}. The 2020 Critical Assessment of Protein Structure Prediction demonstrated AlphaFold's unprecedented success, showcasing its potential to transform structural biology research⁶. This tool provides researchers with a powerful means to explore protein and peptide conformations, offering insights that are critical for drug discovery, disease mechanism elucidation, and synthetic biology^{7–10}.

Self-assembling peptides have emerged as versatile biomaterials with significant potential in various biomedical applications, including tissue engineering, drug delivery, and regenerative medicine^{11–18}. These peptides can spontaneously organize into well-defined nanostructures, such as fibrils, hydrogels, and nanovesicles, driven by non-covalent interactions like hydrogen bonding, hydrophobic effects, and electrostatic interactions. Their inherent biocompatibility, tunable mechanical properties, and ability to mimic natural extracellular matrices make them ideal candidates for creating scaffolds that support cell growth and differentiation. Recent studies have demonstrated the effectiveness of peptide-based hydrogels in 3D cell culture systems, highlighting their role in advancing tissue engineering and regenerative medicine.

Cancer Organoids are three-dimensional cell culture systems that closely mimic the architecture and functionality of real cancer¹⁹. Derived from cell lines or primary tissues, Organoids provide a more physiologically relevant model for studying cancer development, disease pathology, and drug response compared to traditional two-dimensional cultures²⁰. These miniaturized organ systems have revolutionized biomedical research by enabling the study of complex cellular interactions in a controlled environment. Organoids have been successfully used in cancer research, offering insights into disease mechanisms and facilitating the development of personalized medicine strategies²¹.

The primary scientific challenge addressed in this study is the need for an efficient, stable, and standard platform for the 3D culture of tumor cells. Traditional 2D cultures fail to replicate the complex microenvironment of tumors, limiting the accuracy of drug testing and disease modeling²². Developing a stable, biocompatible matrix

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that can support the growth and proliferation of tumor organoids is essential for advancing cancer research and precision medicine²³. By integrating computational predictions with experimental validations, this study aims to create a robust peptide-based hydrogel that facilitates the formation and growth of endometrial cancer organoids, providing a more accurate model for studying tumor biology and testing therapeutic interventions.

Endometrial cancer is the most common gynecological malignancy in developed countries, with rising incidence globally²⁴. It primarily affects postmenopausal women, and its early detection typically results in favorable outcomes due to the localized nature of the disease. However, advanced or recurrent endometrial cancer, particularly cases with resistance to standard chemotherapy, presents significant treatment challenges and is associated with poor prognosis²⁵. Endometrial cancer can be classified into two major subtypes: Type I (endometrioid) and Type II (non-endometrioid). Type I tumors are often hormone-sensitive and linked to excess estrogen, while Type II tumors are more aggressive and less responsive to conventional treatments^{26,27}. Despite advancements in early detection, treatment options for advanced or recurrent endometrial cancer remain limited, with chemotherapy regimens often proving ineffective in patients with drug-resistant disease²⁸. To address these drug-resistance challenges, the use of defined-hydrogel-based tumor organoids has emerged as a powerful, standard, and stable tool for modeling cancer biology and drug response in vitro. A Carboplatin-resistant patient was identified, and tumor tissue was obtained for RFC-based organoid culture. The organoids were subsequently subjected to comprehensive drug sensitivity screening.

Combining AlphaFold's structural prediction capabilities with the self-assembling properties of peptides and the advanced modeling provided by cancer organoids offers a synergistic approach to address complex biological questions. AlphaFold enables precise predictions of peptide structures^{29–35}, guiding the design of peptides that can form stable, functional hydrogels. These hydrogels, in turn, provide an optimal environment for the growth and proliferation of organoids, closely mimicking the native extracellular matrix. This integrated approach enhances the reliability and physiological relevance of organoids cultures, facilitating more accurate studies of disease mechanisms and drug responses. By leveraging the strengths of these cutting-edge technologies, this study aims to develop a powerful platform for cancer research, ultimately contributing to the advancement of precision medicine and personalized therapeutic strategies.

By using AlphaFold, researchers have capacities to predict how specific peptide sequences will fold and interact, enabling the design of peptides with optimal self-assembling properties³⁶. This predictive power is particularly valuable for creating peptide-based hydrogels with precise structural and functional characteristics, ensuring they can effectively mimic the natural extracellular matrix and support 3D cell culture^{37,38}. The resulting peptide hydrogels offer a biocompatible and tunable environment for growing organoids, which are critical for studying complex biological processes and developing personalized medicine strategies.

Furthermore, the integration of AlphaFold predictions with peptide-based hydrogels enhances the efficiency and effectiveness of developing new biomaterials. It reduces the trial-and-error approach traditionally associated with material design, speeding up the process of finding suitable peptide sequences for specific applications. This approach not only saves time and resources but also provides a more systematic and rational method for biomaterial development, leading to more reliable and reproducible results.

Materials and methods

Materials

The Endometrial cancer organoid culture medium was prepared by combining Advanced DMEM/F12 with 10 mM HEPES and 2 mM L-Glutamine. The medium was supplemented with 1% B27 Supplement (Gibco, USA), 1% N2 Supplement (Gibco, USA), 1.25 mM N-Acetylcysteine (Sigma-Aldrich, USA), 10 mM Nicotinamide (Sigma-Aldrich, USA), 500 nM A83-01 (Tocris Bioscience, UK), 10 μ M SB202190 (Sigma-Aldrich, USA), 50 ng/ml EGF (PeproTech, USA), 100 ng/ml FGF10 (PeproTech, USA), 10 ng/ml FGF2 (PeproTech, USA), 500 ng/ml R-spondin 1 (PeproTech, USA), 100 ng/ml Noggin (PeproTech, USA), 10 nM Gastrin (Sigma-Aldrich, USA), 10 μ M Y-27632 (Selleck Chemicals, USA), and 1X Primocin (InvivoGen, USA).

Synthesis of RFC

The sequence of RFC is Ac-Arg-Leu-Asp-Ile-Lys-Val-Glu-Phe-Cys -Arg-Leu-Asp-Ile-Lys-Val-Glu-Phe-Cys-CONH₂ and was synthesized by Shanghai Scipeptide (Shanghai, China).

Prediction of RFC with Alpha fold 3

The predictions for the RFC and its sodium ion-mediated assembly were generated using the AlphaFold3 online server (<https://alphafoldserver.com>). The pLDDT scores were used to assess the confidence of the predicted structures, with high scores confirming the reliability of the α -helical conformation and the positioning of sodium ions within the assembly. The prediction process followed the server's default parameters to ensure reproducibility and standardization. The predicted structures were visualized using ChimeraX. Key structural features, such as the α -helical conformation, hydrogen bonding, and electrostatic interactions, were highlighted. To illustrate the peptide's electrostatic surface potential, a Coulombic coloring scheme was applied in ChimeraX, with regions of negative, positive, and neutral charge annotated. Additionally, the self-assembled network of multiple peptide chains and sodium ions was generated to demonstrate the molecular basis of hydrogel formation.

Preparation of RFC gel

A 100 mg powder of RFC was weighed and filled with 10 ml of ultrapure water to prepare a 10 mg/ml stock solution. Mix 0.6 ml of the stock solution at a concentration of 10 mg/ml with 0.4 ml of PBS to prepare an RFC gel at a concentration of 6 mg/ml. Let the mixture stand for 5 min to stabilize the gel for subsequent experiments. Other concentrations were prepared using the same method.

Congo red staining

0.5 g of Congo red powder was dissolved in 50 ml of 95% ethanol and mixed thoroughly. Then, 50 ml of PBS was added and mixed until the solution was uniform. To enhance the staining efficacy, 0.1 g of lithium carbonate was optionally added. The solution was then filtered to remove any undissolved particles and stored in a light-protected bottle to prevent degradation. Before use, the solution was ensured to be well-mixed to guarantee consistent staining results. Applying the sample gel preparation protocol that we described before as a final concentration of 6 mg/ml, 5 mg/ml, 4 mg/ml, and 2.5 mg/ml, but mixing with Congo red staining buffer instead of PBS.

Circular dichroism (CD) spectroscopy

The CD spectrometer (Jasco J-1500, Japan) was warmed up and set with parameters for far-UV CD (190–260 nm). A baseline spectrum was recorded using the buffer solution in a quartz cuvette and subtracted from the sample spectrum. The protein or peptide solution was then placed in the cuvette, and the CD spectrum was recorded, averaging multiple scans to improve signal-to-noise ratio. The spectra were analyzed to determine the secondary structure content by converting the CD signal to mean residue ellipticity and comparing it to reference data. After measurements, the cuvette was cleaned, and the instrument was properly maintained. The CD data was quantified by BESTSEL.

Atomic force microscopy (AFM)

The RFC was prepared by cleaning and fixing it onto the AFM sample stage. An appropriate AFM probe was installed and aligned with the laser to ensure proper reflection onto the position-sensitive detector. The AFM device (Brooke Multimode8, USA) was initialized, and scanning parameters such as mode (contact mode, tapping mode), range, speed, and resolution were set. The scan was performed, monitoring in real-time to ensure image quality. Upon completion, the data were saved and analyzed using AFM software to determine surface morphology, roughness, and particle size. After scanning, the AFM equipment and probe were cleaned and properly stored.

Transmission electron microscopy (TEM)

The RFC was first fixed with glutaraldehyde and then post-fixed with osmium tetroxide to preserve and enhance contrast. It was dehydrated through a graded series of ethanol or acetone solutions and embedded in epoxy resin, which was polymerized into a solid block. Ultra-thin Sects. (50–100 nm) were cut from the resin block using an ultramicrotome and placed on copper grids. The sections were stained with uranyl acetate and lead citrate for contrast. The TEM (JEM-2100, Japan) was initialized with the appropriate voltage (80–200 kV), and the stained grids were loaded into the instrument. The sample was imaged at various magnifications, and images were captured with a digital camera system for subsequent data analysis with Image J.

Rheometer

The RFC was prepared to ensure uniformity and absence of bubbles and was loaded into the rheometer (TA Instruments AR-G2, USA) using an appropriate measurement system such as a parallel plate or cone plate. The rheometer was initialized, and the measurement system was cleaned and installed. If temperature control was required, the temperature was set and stabilized. The sample was carefully placed in the measurement system, ensuring even coverage and proper gap setting. Experimental parameters, including shear rate, strain amplitude, and frequency range, were configured based on the experimental goals. The test was conducted, recording the rheological responses such as viscosity, storage modulus (G'), and loss modulus (G''). The collected data was analyzed using the rheometer's software to determine the sample's rheological properties. After the experiment, the measurement system was cleaned thoroughly, and routine maintenance was performed on the rheometer to ensure accurate and consistent results.

Primary tissue

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Henan Cancer Hospital Ethics Committee. Informed consent was obtained from all participants or their legal guardians prior to tissue donation and use in this study. The endometrial cancer tissue is first minced into small fragments using sterile surgical scissors. These fragments are then incubated in a digestion buffer, typically containing enzymes collagenase (10 µg/ml) and DNase I (10 mg/ml), at 37 °C with gentle agitation for 30 min. After digestion, the mixture is filtered through a 70 µm cell strainer to remove undigested debris and obtain a uniform single-cell suspension. The cells are then washed with PBS or culture medium and centrifuged to pellet the cells.

3D Culture organoids with RFC

5×10^3 Endometrial cancer cells were resuspended in 50 µl PBS, then mixed with the 50 µl of 6 mg/ml stabilized RFC gel to create a homogeneous cell-gel mixture. This mixture was dispensed into 96 well plates and allowed to solidify completely. After gel formation, add 150 µl of endometrial cancer organoids culture media. The plate was then incubated at 37 °C with 5% CO₂ for the desired period and changed the media every day.

Organoids pathology

To prepare organoids for histological analysis, the organoids were first harvested and embedded in 4% high-melt agarose. Fixed in paraformaldehyde overnight. The solidified agarose blocks containing the fixed organoids were dehydrated through a graded ethanol series (70%, 90%, 100%) and cleared in xylene.

Endometrial cancer organoids drug screening

Drug sensitivity was assessed using the CellTiter-Glo 2.0 assay. Organoids were dissociated into single cells, and 1000 cells per well were seeded in 96-well plates. The cells were treated with seven concentrations of the test drugs, ranging from 10^{-4} to 10^2 μ M for 7 groups, DMSO was used to be negative control. After 48 h of drug exposure, CellTiter-Glo 2.0 reagent was added to each well to assess cell viability based on ATP levels. Luminescence was measured using a microplate reader, and the results were normalized to controls to calculate relative viability at each drug concentration.

Results

AlphaFold predicts sodium ion-mediated α -helical stability and self-assembly of the RFC

The predicted structure of the RFC (Fig. 1A) demonstrates a well-defined α -helix stabilized by sodium ions (Na^+). AlphaFold accurately predicted the characteristic geometry of an α -helix, including the right-handed helical conformation and the regular hydrogen bond pattern between backbone carbonyl oxygen ($\text{C}=\text{O}$) and amide hydrogen ($\text{N}-\text{H}$) of n and $n+4$ residues. The stabilization of the α -helix by Na^+ was evident through electrostatic interactions with negatively charged residues, specifically glutamate (Glu) and aspartate (Asp), as shown by the red dashed lines in the structure. This interaction neutralized the negative charges, reducing electrostatic repulsion and promoting structural stability.

The self-assembly model of ten peptide chains with forty sodium ions (Fig. 1B) revealed a parallel alignment of peptides, forming a scaffold-like network. Sodium ions played a critical role in facilitating this arrangement by acting as charge bridges, linking the negatively charged residues across adjacent peptide chains. This ion-mediated interaction enabled the peptides to overcome electrostatic repulsion and assemble into a stable higher-order structure.

Hydrogen bond analysis further confirmed the structural stability of the α -helix (Fig. 1C). The intramolecular hydrogen bonds between n and $n+4$ residues contributed significantly to the preservation of the helical conformation. These hydrogen bonds, combined with Na^+ -mediated charge neutralization, provided a dual mechanism for stabilizing the peptide structure.

Electrostatic surface potential analysis (Fig. 1D) highlighted the spatial distribution of charges across the peptide. Negatively charged regions, represented in red, corresponded to Glu and Asp residues, which served as primary binding sites for sodium ions. In contrast, positively charged regions (blue) were concentrated near the N-terminal, while neutral regions (white) likely corresponded to hydrophobic residues, such as leucine (Leu) and valine (Val). This distinct charge distribution underscores the role of electrostatic interactions in peptide stabilization and network assembly. Additionally, the presence of hydrophobic residues on the surface suggests a potential contribution of hydrophobic interactions in driving peptide aggregation.

The combination of these structural features, including α -helical geometry, sodium ion coordination, charge distribution, and hydrophobicity, underscores the versatility of RFC in forming a stable hydrogel under physiological conditions. The predictive accuracy of AlphaFold was instrumental in elucidating these structural details, providing insights into the molecular mechanisms of peptide stability and self-assembly. These findings suggest that RFC could serve as a robust scaffold for hydrogel formation, with potential applications in biomaterials and tissue engineering.

Circular dichroism spectrum validates α -helical stability in the RFC

The predicted structure of the peptide sequence using AlphaFold indicated a predominantly helical conformation. The contact maps showed close inter-residue contacts, and the predicted IDDT scores were high, suggesting a stable and reliable helical structure. The visual representation confirmed the helical nature, with high confidence in the predicted positions of the residues. To validate the AlphaFold predictions, Circular Dichroism (CD) spectroscopy was employed to analyze the peptide's secondary structure. The CD spectrum displayed characteristic peaks indicative of α -helical content, with a positive peak near 190 nm and a negative peak around 210–220 nm (Fig. 2A). The secondary structure content derived from the CD data revealed that the peptide consists of 90.20% regular α -helix, 5.00% distorted α -helix, and 4.80% parallel β -sheet. This high helical content aligns well with the AlphaFold prediction, confirming that RFC predominantly forms a stable α -helical structure in solution. This integrated analysis demonstrates the utility of combining computational and experimental approaches to explore peptide structures (Fig. 2B).

Self-assembly of the RLDIKVEFRLDIKVEFCC peptide into a nanofiber network

The AFM images reveal a network of fibrillar structure, indicating the peptide's propensity to form well-defined fibrils (Fig. 3A). The TEM images further confirm the fibrillar nature of the peptide aggregates (Fig. 3B). The consistent observation of fibrils in both AFM and TEM analyses underscores the stability and robustness of these structures. The fiber range is 15 ± 4 nm ($n = 50$). These findings are in line with the AlphaFold and CD spectroscopy results, which suggested a stable helical structure, likely contributing to the formation of these fibrillar assemblies. This integrated analysis demonstrates the peptide's ability to form stable, organized fibrils, providing a foundation for further functional studies.

RFC hydrogel exhibits strong elastic behavior under dynamic rheology

In the frequency sweep data, the storage modulus (G') is consistently higher than the loss modulus (G'') across the entire frequency range tested (Fig. 4A). This indicates that the peptide gel exhibits predominantly elastic behavior, characteristic of a well-formed gel network. The G' value remains relatively stable and higher than G'' , suggesting that the gel maintains its structural integrity and elasticity over the tested frequency range. The difference between G' and G'' indicates that the gel has a solid-like behavior with a dominant elastic component, which is typical for gels that form stable three-dimensional networks, suggesting that the peptide forms a stable,

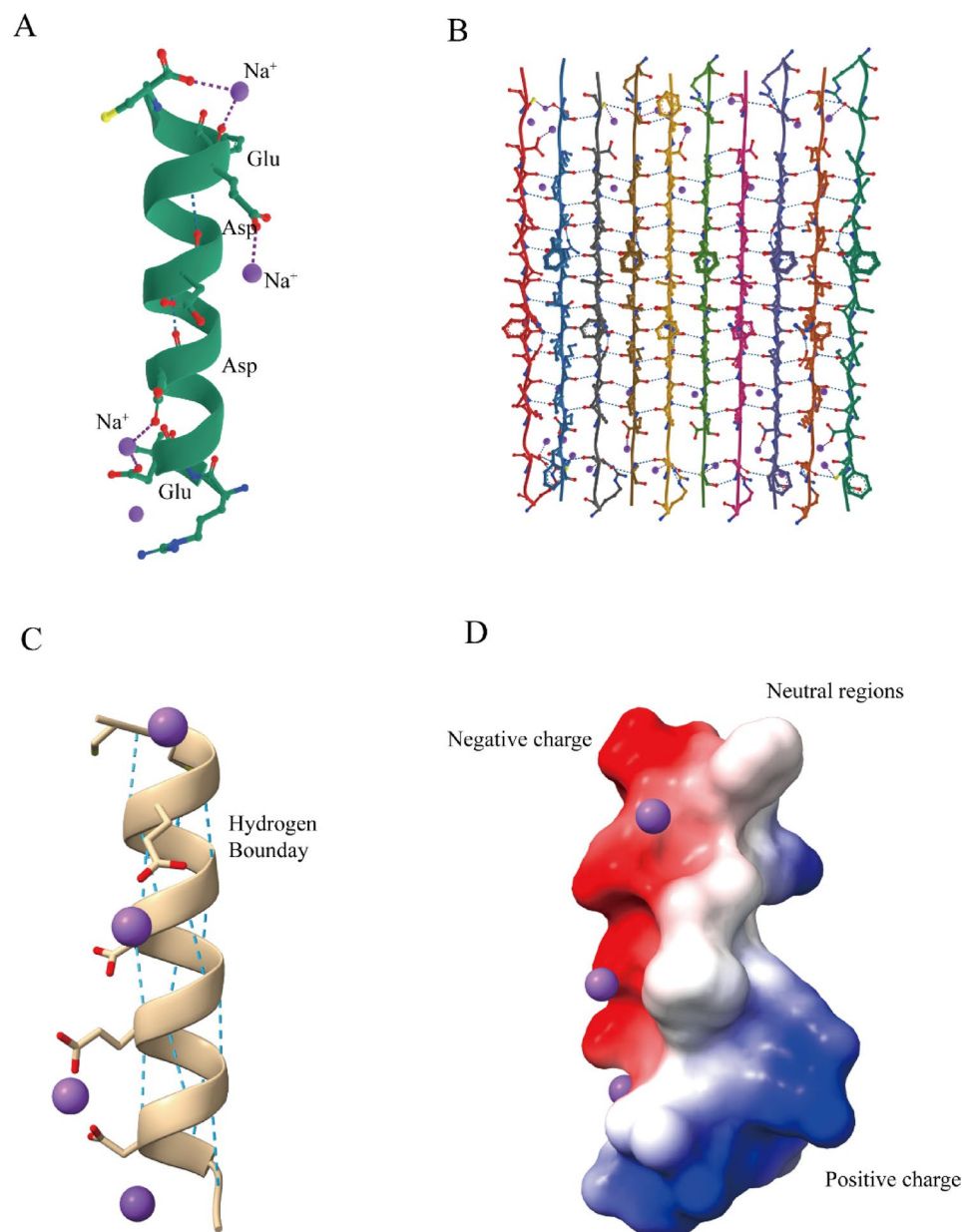


Fig. 1. Sodium ions stabilize the helical structure of the RFC and promote self-assembly. (A) AlphaFold-predicted structure of the RFC with four sodium ions (Na^+), showing sodium ions interacting with Glu and Asp residues through electrostatic interactions (red dashed lines). (B) Predicted self-assembly model of ten RFC chains and forty sodium ions. The peptides align in a parallel arrangement, forming a scaffold-like network. (C) Hydrogen bond analysis of the RFC reveals intramolecular hydrogen bonds (blue dashed lines), contributing to the stabilization of the helical structure. (D) Electrostatic surface potential of the RFC, highlighting negatively charged (red), positively charged (blue), and neutral regions (white), which correspond to potential interaction sites for sodium ions and other molecules.

elastic gel network, making it a potential candidate for various biomedical and biotechnological applications, align with 3D cell culture, drug delivery, and wound healing.

Concentration-dependent gelation of the RFC

The Congo red staining and gravity-induced flow assessment collectively demonstrate that the peptide gel's structural integrity and stability are highly dependent on concentration (Fig. 5A). Higher concentrations (6 mg/ml and 5 mg/ml) form robust, stable gels with strong fibrillar networks that resist gravitational flow (Fig. 5B). In contrast, lower concentrations (4 mg/ml and 2.5 mg/ml) result in weaker gels with less dense fibrillar structures

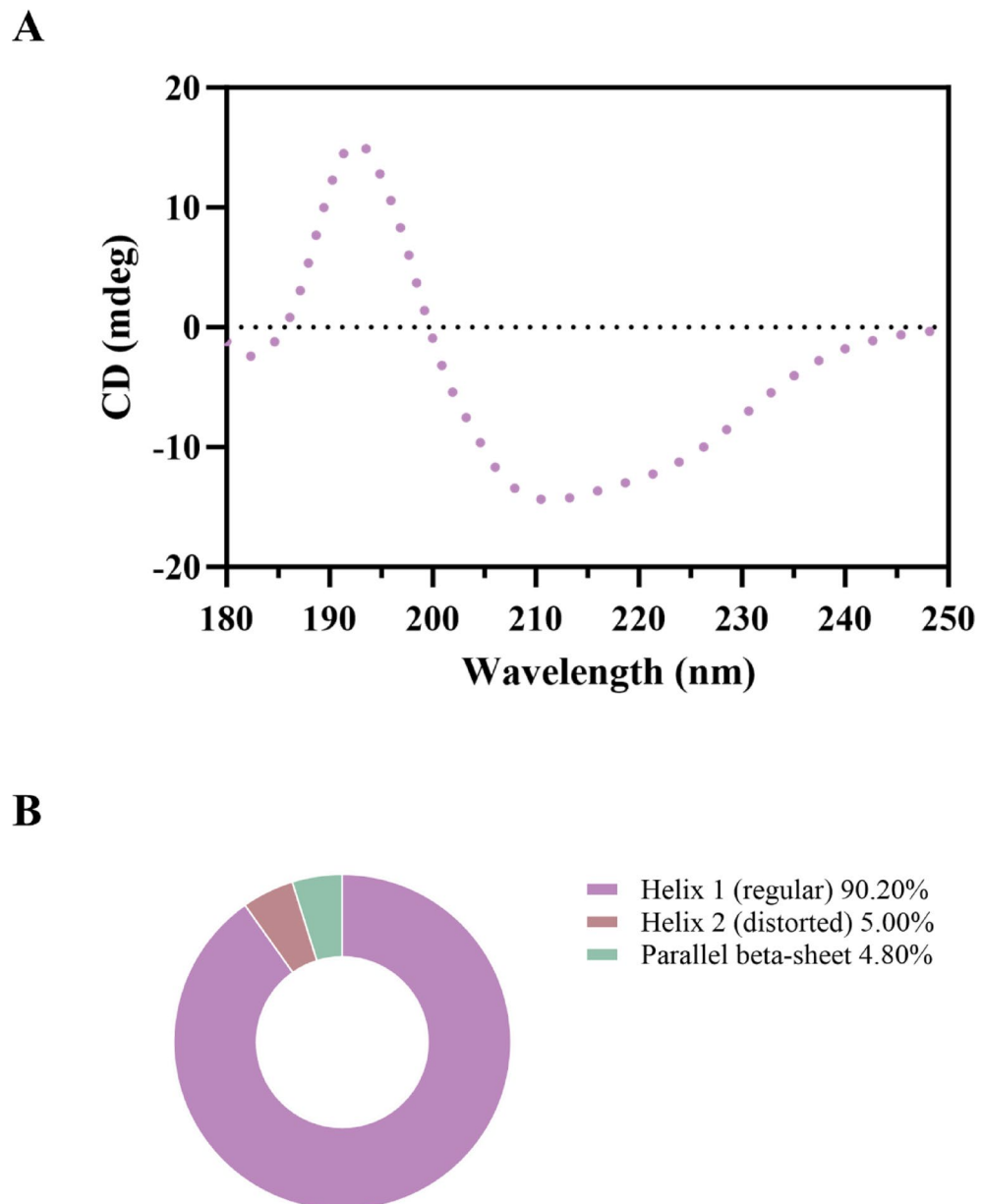


Fig. 2. CD analysis confirms the predominant α -helical structure of the peptide. (A) CD spectrum of the RLDIKVEFRLDIKVEFCC peptide. The spectrum shows a characteristic positive peak near 190 nm and a negative peak between 210–220 nm, indicative of α -helical content. (B) Quantitative analysis of secondary structure composition derived from the CD data. The pie chart illustrates the distribution of secondary structure types: 90.20% regular α -helix, 5.00% distorted α -helix, and 4.80% parallel β -sheet.

but homogeneity, which are more prone to flow under gravity. These findings are consistent with the rheological data, further confirming the concentration-dependent mechanical properties of the peptide gels. In contrast, a concentration of 6 mg/ml is too viscous, potentially causing cell compression and nutrient deprivation, leading to cell death. Therefore, the 4 mg/ml gel demonstrates more uniform staining and is more suitable for 3D cell culture, facilitating better medium infiltration. Therefore, we chose a concentration of 4 mg/ml as the final concentration for cell culture.

3D culture endometrial cancer organoids with RFC

The peptide gel successfully facilitated the 3D culture of endometrial cancer organoids, demonstrating its potential as a viable matrix for tumor cell growth and proliferation (Fig. 6A). The significant increase in organoid size over the 14-day period highlights the gel's effectiveness in supporting long-term cell viability and growth. This capability to maintain and expand organoids in a 3D culture environment is crucial for various applications, including cancer research, drug screening, and understanding tumor biology with defined and clear peptide-based hydrogel. The organoids display a disorganized, hypercellular architecture with nuclear pleomorphism,

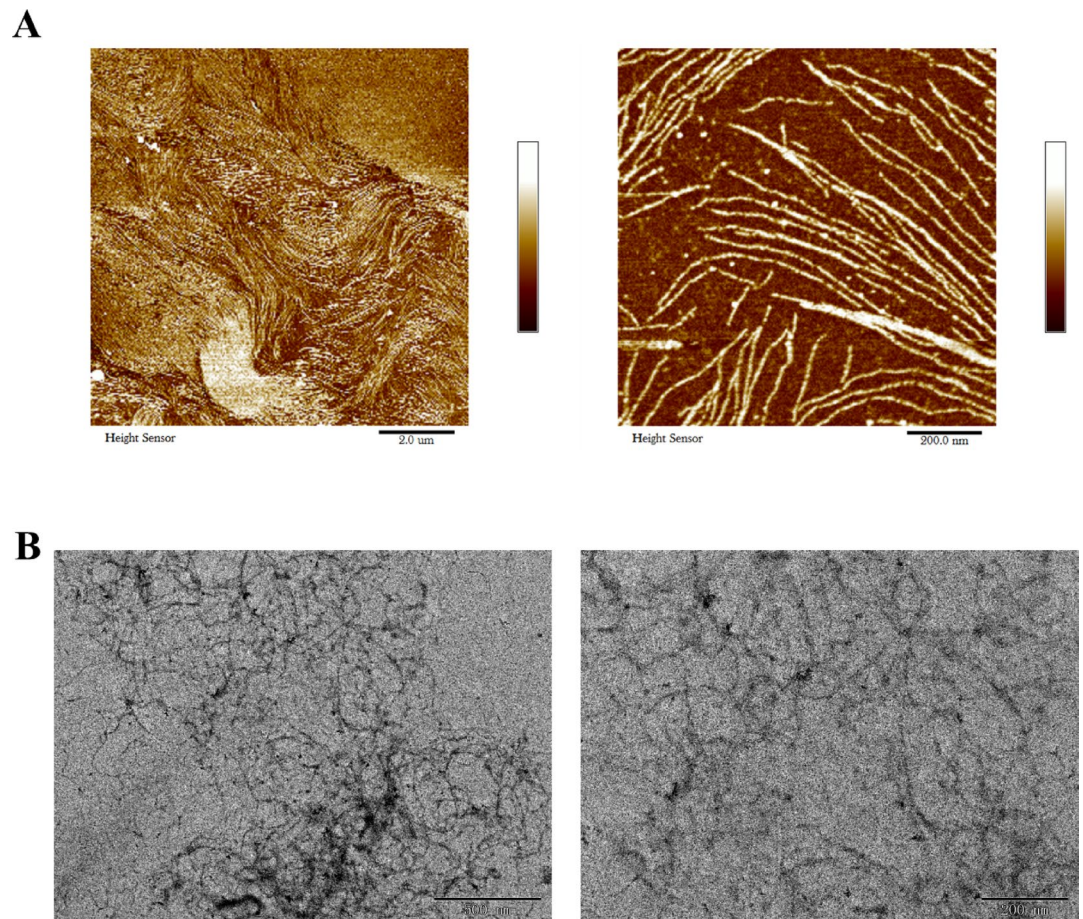


Fig. 3. Formation of a nanofiber network by the RFC (A) AFM images of the peptide (B) TEM images of the RFC.

which is characteristic of malignant endometrial tissue in H&E staining (Fig. 6B). The lower panel shows the native endometrial cancer tissue, with comparable features such as irregular glandular structures and increased nuclear atypia. This indicates that the organoid model faithfully recapitulates the histopathological features of endometrial cancer. The organoids demonstrate extensive Ki-67 positivity, reflecting the aggressive proliferative capacity of endometrial cancer cells. The tissue sections similarly show widespread Ki-67 expression, indicating that the organoids closely mimic the proliferative behavior of endometrial cancer *in vivo* (Fig. 6C).

Dose–response analysis reveals therapeutic strategies for Carboplatin-resistant endometrial cancer

The dose–response analysis of the Carboplatin-resistant endometrial cancer provides critical insights into potential therapeutic strategies. The results indicate that similar to platinum-based agents such as Cisplatin and Carboplatin, other chemotherapeutic drugs tested, including Paclitaxel, Topotecan, and Gemcitabine, show limited effectiveness, with only modest reductions in cell viability (Fig. 7A). These findings suggest that the organoids exhibit a broad resistance to multiple agents, highlighting the challenges of treating this particular cancer. However, one drug, Doxorubicin, stands out as an exception. The steep decline in cell viability with Doxorubicin treatment indicates that the organoids are highly sensitive to this drug. This significant sensitivity positions Doxorubicin as a potentially effective therapeutic option for this platinum-resistant patient, offering a new avenue for clinical intervention. Given the overall resistance to most other drugs in this screening, the high sensitivity to Doxorubicin is particularly promising. It suggests that Doxorubicin could overcome the resistance mechanisms that limit the effectiveness of both platinum-based therapies and other tested agents. Therefore, incorporating Doxorubicin into the patient's treatment plan may provide a more targeted and effective approach, offering hope for better clinical outcomes in managing this challenging case of endometrial cancer.

Discussion

The RFC was thoroughly investigated using a combination of computational and experimental methods. AlphaFold, a state-of-the-art deep learning-based tool, provided high-confidence predictions of the peptide's structure, indicating a predominantly helical conformation. The reliability of these predictions was supported by high predicted IDDT scores and consistent inter-residue contact patterns. Such computational tools represent a

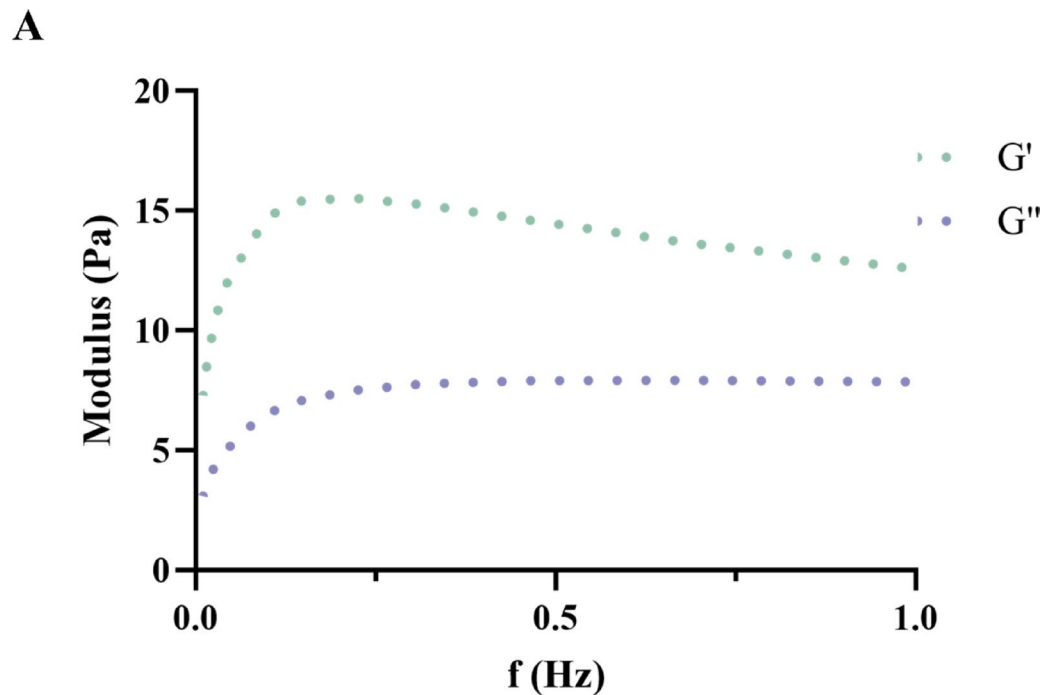


Fig. 4. RFC forms a viscoelastic and predominantly elastic hydrogel (**A**) Frequency sweep of the peptide gel showing the storage modulus (G') and loss modulus (G'') as a function of frequency (f). The storage modulus (G') and loss modulus (G'') provide information on the elastic and viscous properties of the gel, respectively.

significant advancement in structural biology, enabling accurate predictions that are essential for understanding protein function and interactions without the need for labor-intensive experimental methods^{39–41}. The AlphaFold predictions were validated by Circular Dichroism (CD) spectroscopy, which confirmed the helical nature of the peptide. The CD spectrum displayed characteristic peaks for alpha-helices, and the quantitative analysis revealed a substantial helical content (90.2% regular alpha-helix). This experimental confirmation underscores the accuracy of AlphaFold's predictions and highlights the utility of integrating computational and experimental approaches in structural biology.

The peptide forms a stable and organized fibrillar network, essential for its functionality as a hydrogel⁴². A predominantly elastic behavior characteristic of a stable gel network, This mechanical stability is crucial for various applications, including tissue engineering and drug delivery, where consistent structural integrity is required^{43,44}. The integration of AlphaFold for structural predictions, coupled with advanced experimental techniques such as CD spectroscopy, AFM, TEM, and rheological testing, exemplifies the forefront of modern biomedical research. These combined approaches provide comprehensive insights into the structural and functional properties of biomolecules, facilitating the development of novel materials and therapeutics. The peptide gel's application in 3D cell culture further demonstrates the convergence of computational and experimental methodologies, paving the way for innovative solutions in tissue engineering, regenerative medicine, and cancer research. The study of platinum-resistant endometrial cancer cultured in hydrogel reveals important insights into both the growth patterns of the tumor cells and their response to various chemotherapeutic agents. The organoids cultured over 14 days showed increasing complexity and structural organization, suggesting that the hydrogel system provides an optimal microenvironment for tumor proliferation and development. The similarity between organoid structures and actual endometrial cancer tissue, as demonstrated by H&E and Ki-67 staining, further validates the organoid model as a relevant platform for studying tumor biology and drug response. Drug screening using dose–response curves highlights a broad resistance of the tumor organoids to most tested chemotherapeutics, including platinum-based agents (Cisplatin, Carboplatin), which is consistent with the clinical profile of the patient being resistant to platinum drugs. Other agents like Paclitaxel, Topotecan, and Gemcitabine also showed limited effectiveness, as indicated by their relatively shallow dose–response curves and modest impact on cell viability. These results suggest that the cancer cells have developed resistance mechanisms that affect a broad range of drugs, posing a significant challenge in identifying effective therapies. We acknowledge that intact 3D organoids would provide a more physiologically relevant system for drug screening. However, global standardization of organoid size and morphology remains a challenge, and many studies therefore employ dissociation into single cells to ensure assay reproducibility and cost-effectiveness. Although dissociated cells lose the spherical morphology of organoids, previous studies have shown that they retain key genetic and phenotypic characteristics of the original organoids, and even the ability to reform organoid structures. In this study, we therefore chose the single-cell format to enable standardized dose–response testing, while using the RFC hydrogel scaffold to demonstrate the feasibility of AlphaFold-predicted peptides in supporting organoid culture. Future work will leverage our recently developed micromold system to generate uniform organoids and

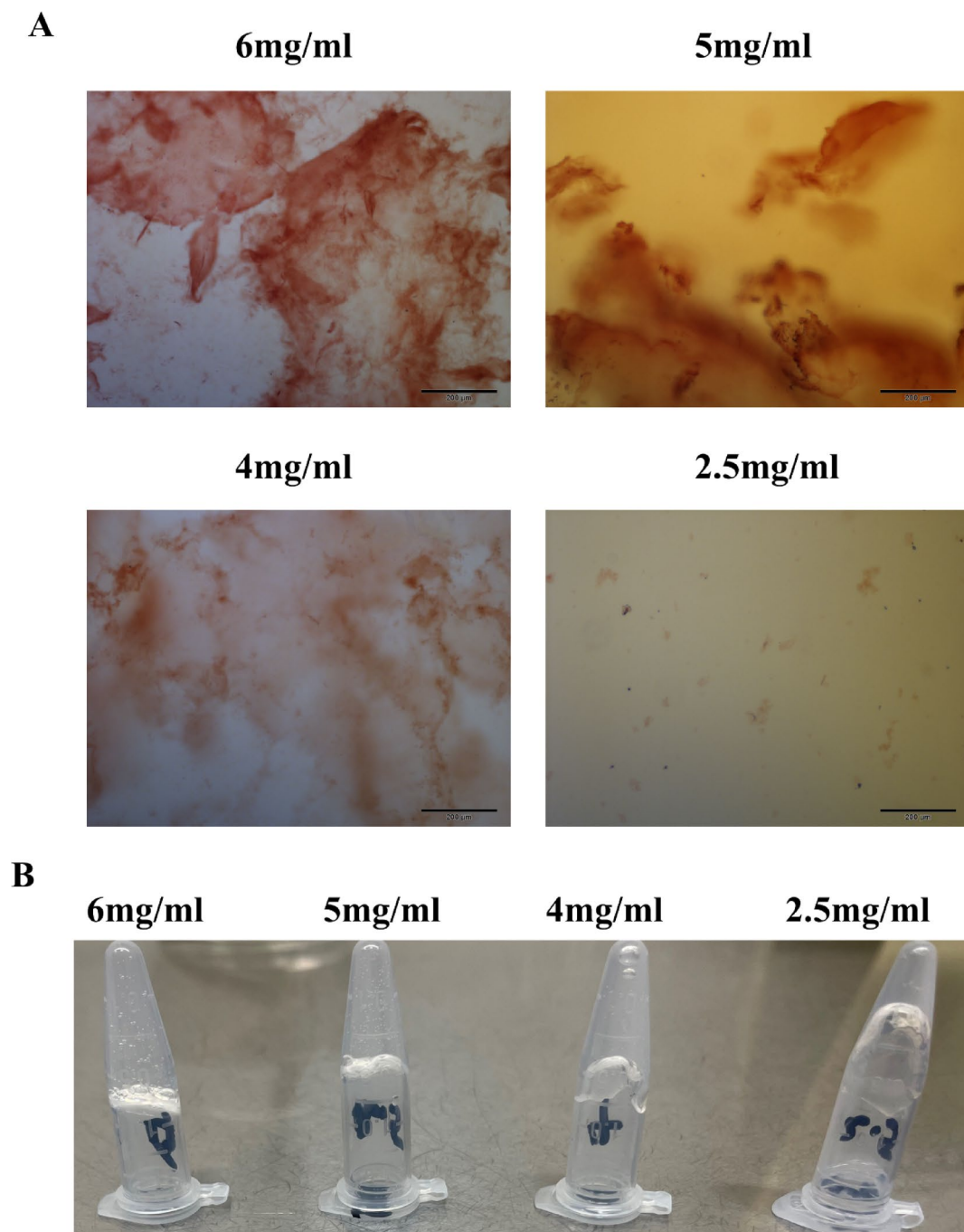


Fig. 5. Concentration impacts the network structure and gelation properties of the peptide hydrogel. **(A)** Microscopic images of peptide gels stained with Congo red at concentrations of 6 mg/ml, 5 mg/ml, 4 mg/ml, and 2.5 mg/ml. **(B)** Photographs of peptide gels at the same concentrations (6 mg/ml, 5 mg/ml, 4 mg/ml, and 2.5 mg/ml) placed upside down to assess the impact of gravity on the gel's structural integrity and flow resistance.

perform drug screening directly in intact 3D structures. Organoids represent cutting-edge tools in precision medicine, offering personalized models for studying disease mechanisms and testing therapeutic responses. The successful culture of endometrial cancer organoids using the peptide gel highlights its potential for creating patient-specific models, enabling tailored treatment strategies. This approach aligns with the growing emphasis on precision medicine, where treatments are customized based on individual patient profiles, leading to improved outcomes and reduced side effects⁴⁵. In addition, while our observations showed that RFC hydrogels maintained structural integrity in PBS at 37 °C for at least three weeks, systematic evaluation of enzymatic degradation remains an important future direction. Such studies will be particularly relevant if RFC hydrogels are to be developed further as wound repair scaffolds or as novel soft tissue filler materials for aesthetic applications.

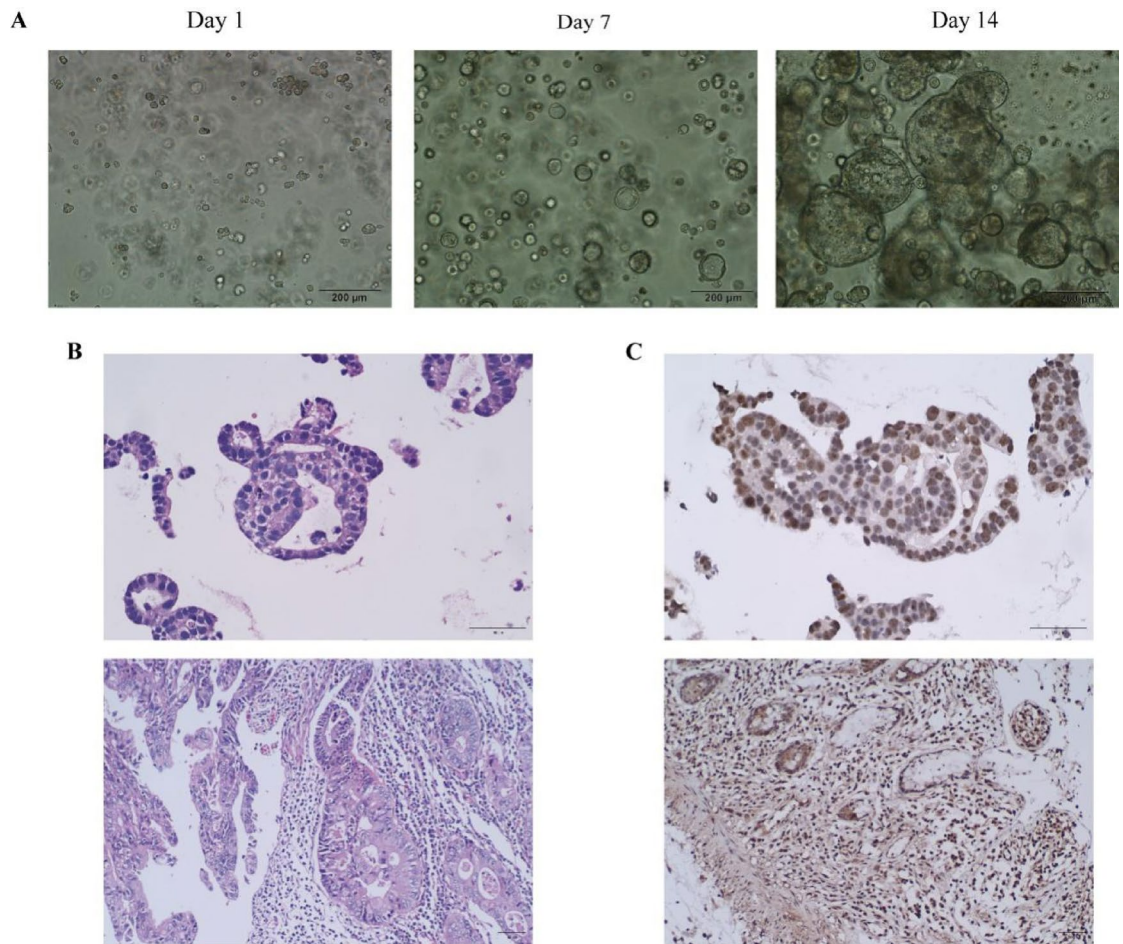


Fig. 6. Growth and tissue-like properties of organoids cultured in RFC hydrogel. **(A)** Representative phase-contrast images of organoids cultured in hydrogel over 14 days. Images are shown from day 1, day 7, and day 14, highlighting the progressive growth and morphological changes. Scale bar: 200 µm. **(B)** Hematoxylin and eosin (H&E) staining of organoids (top) and tissue sections (bottom), showing structural similarities between organoids and their tissue counterparts. Scale bars: 50 µm (top) and 100 µm (bottom). **(C)** Ki-67 immunohistochemistry staining of organoids (top) and tissue sections (bottom) to assess proliferative activity. Scale bars: 50 µm (top) and 100 µm (bottom).

Conclusion

The comprehensive analysis of the RFC, utilizing AlphaFold, CD spectroscopy, AFM, TEM, rheological testing, and Congo red staining, demonstrated its predominantly helical structure and robust mechanical properties. The AlphaFold predictions, validated by CD spectroscopy, revealed a high alpha-helical content, while AFM and TEM analyses confirmed the formation of a dense fibrillar network, particularly at higher concentrations. Rheological measurements indicated a stable gel network with predominantly elastic behavior, further supported by Congo red staining and gravity-induced flow assessments that highlighted the concentration-dependent structural integrity of the peptide gel. Additionally, the peptide gel successfully facilitated the 3D culture of endometrial cancer organoids, showing significant growth over 14 days and underscoring its potential as a biomimetic matrix for tissue engineering and cancer research. The combination of phase-contrast imaging, H&E staining, and Ki-67 immunohistochemistry highlights the structural and functional similarities between the organoid model and the original endometrial cancer tissue. These findings underscore the value of using organoids as a patient-specific model for studying tumor behavior and drug response. The analysis of endometrial cancer cultured in hydrogel and their response to chemotherapeutics underscores the complexity of treating platinum-resistant cancers. While most of the tested drugs, including Cisplatin, Carboplatin, and Paclitaxel, show limited efficacy in this model, Doxorubicin demonstrates significant sensitivity. This finding presents Doxorubicin as a viable alternative for treating this patient, offering a new direction for clinical intervention. The data suggest that focusing on Doxorubicin, potentially in combination with other therapeutic approaches, may improve outcomes for patients with platinum-resistant endometrial cancer. This model also underscores the importance of personalized drug screening in identifying effective treatments tailored to individual patients' cancer profiles. This integrated approach combining computational predictions with experimental validation provides a

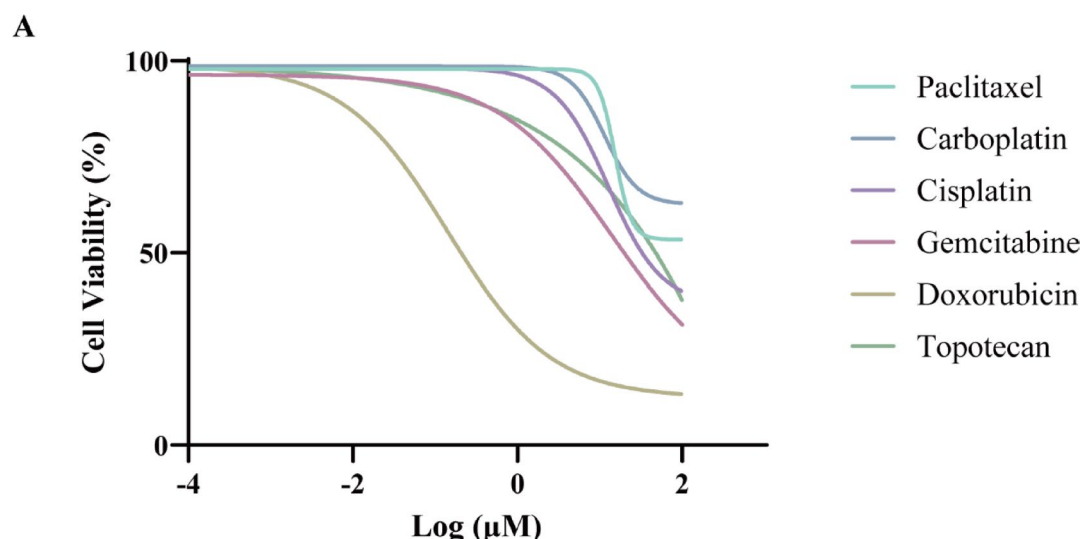


Fig. 7. Doxorubicin shows the highest efficacy among chemotherapeutic agents on endometrial cancer organoids cultured in RFC hydrogel. (A) Cell viability was assessed after treatment with increasing concentrations of Paclitaxel, Carboplatin, Cisplatin, Gemcitabine, Doxorubicin, and Topotecan.

comprehensive understanding of the peptide's properties and demonstrates its suitability for various biomedical applications, particularly in precision medicine and the development of personalized therapeutic strategies.

Data availability

All data generated or analyzed during this study are included in this published article.

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Author contributions

M.Z. conceived the study, designed the experiments, and wrote the initial draft of the manuscript. Y.W. performed data collection, conducted statistical analysis, and contributed to the interpretation of the results. D.L. prepared Figures, conducted literature review, and revised the manuscript for intellectual content. All authors discussed the results, contributed to the final manuscript, and approved its submission.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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