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Janus Nanoparticles Loaded 3D Bio Printed Scaffold as a Dual Drug Delivery System

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Abstract: Herein, a new type of controlled-release system is fabricated using polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles for the creation preparation study. They include doxorubicin (DOX), a standard chemical for chemotherapy, and curcumin, part of the plant turmeric. The material is produced as a dual-loaded nanoparticle system, where the widely used chemotherapeutic drug Doxorubicin (DOX) and the commonly known anti-inflammatory/antioxidant Curcumin are encapsulated in their respective phases, PCL and PLGA. Next, the Janus NPs were loaded into a 3D bioprinted support matrix composed solely of Carbopol. The design of this scaffold was engineered to provide a biocompatible and supportive environment for the controlled release of medication over an extended period. The biocompatibility, kinetics of drug release, and biomechanical properties were also analyzed in the 3D-printed scaffold. The structural integrity of the scaffold was strengthened, and DOX as well as curcumin can be continuously released for long periods by using Janus nanoparticles. Moreover, dual drug-loaded nanoparticles provided a sustained release plan that could be an innovative technique for improved anticancer activity in synergy therapy; the scaffold supported particle stability as indicated by favorable results of preliminary research. This system might offer a more potent and target-oriented strategy in cancer therapy through the sustained release of curcumin and DOX, effectively reducing cancer cell viability compared to conventional drug delivery approaches.

Keywords: Janus nanoparticles, 3D bioprinting, Drug delivery

Introduction

Each year millions of new cases are identified, making cancer one of the most significant global health issues. Although significant strides have been made in the scientific understanding of cancer, treating it remains a daunting proposition fraught with the enervating attributes of conventional therapeutic modalities. Chemotherapy, one of the more common cancer treatments, has been effective in controlling or curing some cancers. However, its application might lead to severe side effects, including non-specific targeting, systemic toxicity, and resistance occurrence, which reduce the therapeutic effectiveness of this drug and increase patient morbidity. These challenges highlight an imminent need for more sophisticated precision and targeted drug delivery strategies to perpetuate treatment efficacy and ameliorate side effects. In the modern era, nanotechnology can offer solutions to these problems through the development of nanoparticle-based drug

delivery systems. Not only does modifying nanoparticles improve a drug's solubility, stability, and bioavailability, but it also better directs the medication to cancerous regions. Janus NPs have one of the most unique biphasic structures, making them an interesting subset among other NP types. These nanoparticles have opposite top and bottom surfaces to which different functionalities can be added. Named after the two-faced Roman god Janus, their structural flexibility provides an attractive option for combinational cancer therapy by carrying multiple therapeutic drugs with diverse physicochemical properties and modes of action.

A novel drug delivery system is developed, fabricated, and described in this study, using polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles. Due to their biocompatibility, tunable degradation rates, and ability to encapsulate a broad range of therapeutic agents, PCL and PLGA have been extensively studied for drug delivery applications. In this system, the Janus nanoparticles have dual therapeutic components with different and complementary anticancer activities: curcumin (CUR) and doxorubicin (DOX). The turmeric plant produces a polyphenol known as curcumin, which has long been notable for its anti-inflammatory, antioxidant, and antimicrobial effects. It has been shown to induce apoptosis, inhibit cancer cell proliferation, and enhance the effects of other chemotherapeutic drugs. However, the application of curcumin has been limited due to its poor solubility in water and susceptibility to degradation. In contrast, doxorubicin (DOX) is a widely used chemotherapeutic drug effective against various malignancies but is associated with severe adverse effects, including cardiotoxicity. To address the limitations of each therapeutic agent, DOX is encapsulated into PLGA, and curcumin is loaded onto PCL within the Janus nanoparticles, creating a dual-agent system with different mechanisms of action. This system is further enhanced by integrating these Janus nanoparticles into a 3D bioprinted scaffold matrix composed solely of Carbopol, which provides a biocompatible and supportive environment for the controlled release of encapsulated drugs. Carbopol, a synthetic polymer, is well-known for its stability and gel-forming properties, making it an ideal material for this scaffold. The present study details the mechanical behavior, biocompatibility, and drug release kinetics of this 3D bioprinted scaffold. Due to the higher stability of the scaffold combined with Janus nanoparticles, curcumin, and DOX can diffuse out steadily onto a solid substrate. The drug release profile was sequential, with DOX following curcumin. This sequential release is beneficial for synergistic cancer therapy since curcumin can sensitize the effect of DOX in killing cancer cells, potentially contributing to a more substantial therapeutic outcome. The study suggests that the scaffold enabled a controlled and prolonged release of curcumin as well as DOX. Furthermore, the results of this study suggest that an approach using a Janus-nanoparticle-based dual payload on 3D bio-printed scaffolds may lead to a long-term reduction in cancer cell viability by releasing drugs over extended periods, potentially offering a more targeted and efficient strategy compared with traditional drug delivery methods. The findings of this study provide insights into how this innovative drug delivery method may help overcome limitations associated with existing cancer therapies, bringing researchers closer to more personalized and definitive clinical strategies. This study offers a comprehensive overview of the original combination of Janus nanoparticles and 3D-printed drug delivery systems, highlighting its potential to enhance treatment efficacy and improve the quality of life for cancer patients by overcoming major challenges in anticancer therapy, including drug solubilization, protection from degradation before entering blood circulation, and specific targeting."

Method

Preparation of Janus Particles

Preparation of the First Emulsion (W1/O):

An aqueous phase (W1) was obtained by dissolving a hydrophilic drug (curcumin) in deionized water. In the second step, to produce organic phase (O) PLGA and PCL were each dissolved separately in dichloromethane (DCM). Then, to form primary emulsions (W1/O), the aqueous phase (W1) was dropwise added into the organic phase under stirring at 4000 rpm. The liquid was also emulsified with ultrasonication to form fine droplets. Following these steps, the sonication process will take two to five minutes depending on the desired droplet size.

Preparation of the Second Emulsion (W1/O/W2):

The external aqueous phase (W2) was prepared by dissolving polyvinyl alcohol (PVA) as an aqueous solution in deionized water. Thereafter, the first emulsion (W1/O) was added to the PVA solution while stirring at a high rate. The second emulsion (W1/O/W2) is composed of an organic phase containing PCL and PLGA spread in

the external aqueous solution forming. The second emulsion was prepared similarly using an ultrasonicator, yet again to ensure a uniform and regular (stable) final product. This sonication step enhances the encapsulation efficiency of drugs and decreases particle size further.

Solvent Evaporation:

The double emulsion (W1/O/W2) was then stirred at room temperature for several hours (4–6 h, typically), allowing the DCM to evaporate. Since the organic solvent evaporated, Solid Janus particles of PLGA/PCL are formed. Stirring was continued until the smell of DCM evaporating completely disappeared.

Particle Collection and Washing:

Janus particles produced were collected by centrifugation at 10,000 rpm for 10 min. Particles were washed to free the suspension of any remaining PVA and unincorporated drug after centrifuging particles at 2000 rpm for 10 min, ejecting out supernatant. Great care was taken to remove all contaminants, and the washing process involved three ‘washes.

Particle Freeze-Drying:

This was followed by drying of washed PLGA/PCL Janus particles in a small amount of deionized water (placed again under vacuum) and subsequent freeze-drying. Most of the time, you can expect it to take 24-48 hrs depending on how much suspension is being dried and the load configuration for your freeze dryer.

Characterization of the Janus Particles:

Janus particle drug loading efficiency and release kinetics after preparation were assessed. The method should provide a complete guide for preparing PLGA/PCL Janus particles using the double emulsion technique, which is capable of encapsulating both hydrophilic and hydrophobic drugs.

Preparation of Bioink for 3D Bioprinting

Formulation of Bioink:

For bioink preparation, Carbopol powder was dissolved in deionized water to produce a 0.5% (w/v) Carbopol solution. As a viscosity enhancer to ensure the homogeneity of the bioink and to achieve the desired consistency for the 3D bioprinting process, Carbopol alone was used, ensuring a smooth and consistent gel formation.

Nanoparticle Incorporation:

PLGA/PCL Janus nanoparticles loaded with CUR & DOX were distributed in the bioink and continuously stirred to create a homogeneous suspension.

3D Bioprinting Process

A 3D bioprinter INKREDIBLE+ was used with an extrusion-based printhead on a syringe platform. The bioink was then syringed into a chamber and extruded through silicon-based nozzles to construct 3D structures layer by layer. The printing parameters, such as pressure, speed, and layer height, were optimized for resolution and structural integrity. These 3D structures were subsequently crosslinked by submerging them in a 100 mM calcium chloride solution post-printing to ensure the mechanical stability of the printed construct

Evaluation of Drug Release and Biocompatibility

In Vitro Drug Release Studies

At 37°C, the printed structures were submerged in phosphate-buffered saline (PBS). With the use of UV-Vis spectrophotometry, the release of doxorubicin and curcumin was tracked throughout time.

Data Analysis

Statistical Analysis

Every experiment was carried out three times, and the results were shown as mean ± standard deviation (SD). One-way ANOVA with post-hoc analysis was used to establish statistical significance ($p < 0.05$ was deemed significant).

Results and Discussion

Characterization of PLGA/PCL Janus Nanoparticles

Zeta Potential and Morphology

In the inverted microscope image, two hemispherical patches are shown corresponding to PLGA and PCL suggesting effective synthesis (Fig.1). The zeta potential of Janus nanoparticles was -25 mV, suggesting a moderate surface charge (Fig.2) This negative charge is useful, as in the end it stabilizes colloidal particles there and does not aggregate in the bioink matrix.

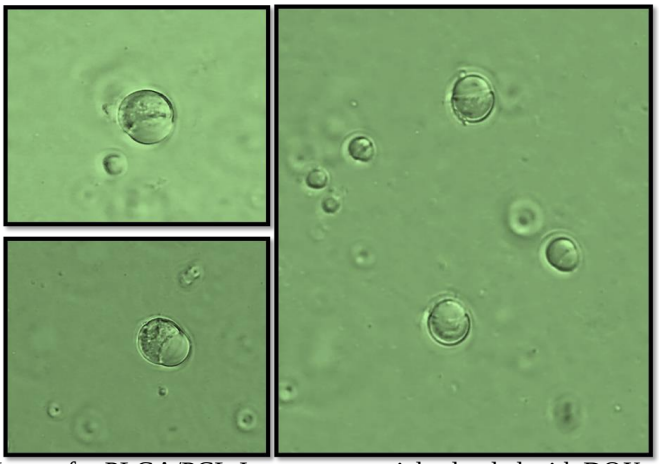

Figure 1. Image for PLGA/PCL Janus nanoparticles loaded with DOX and CUR

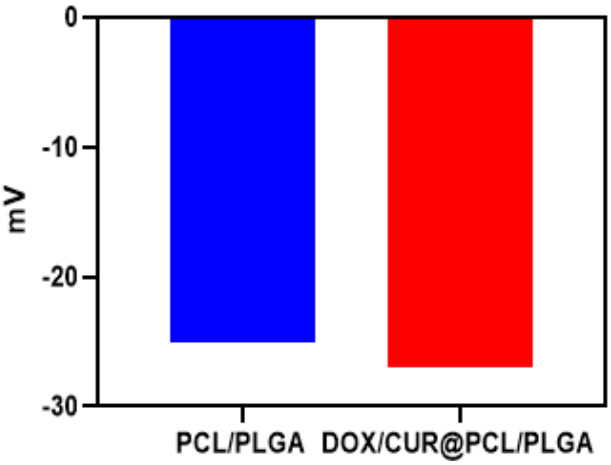

Figure 2. Zeta potential for PCL/PLGA Janus nanoparticles

Drug Encapsulation Efficiency

The enhanced bioink enabled high resolution and fidelity 3D bioprinting of different geometries such as grid, and circular/cylindrical shapes. The structures were crosslinked post-printing in a 0.6M calcium chloride solution to add mechanical strength, thus safeguarding the shape and integrity of objects after they had dissolved away all the PVA.

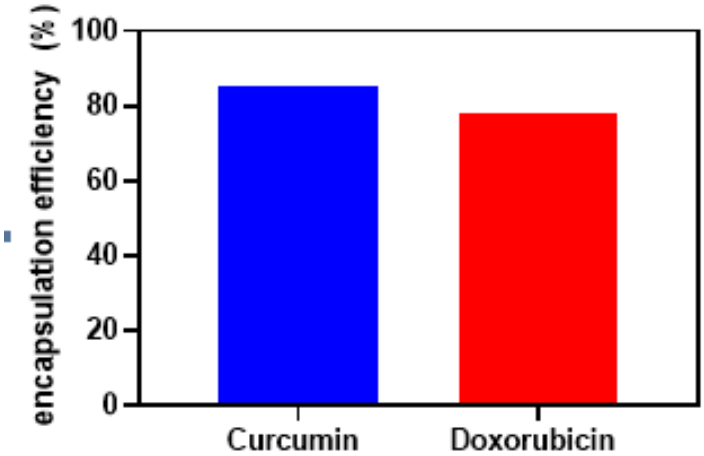

Figure 3. Encapsulation efficiency of CUR and DOX @ PLGA/PCL

Characterization of 3D Bioprinting of Constructs

Printability and Structural Integrity

The aqueous printable matrix was designed to print high-resolution and fidelity arbitrary geometries, like grids or cylinders (which imitate organoids) in 3D. Mechanical stiffness was enforced after printing with additional post-processing crosslinking in a calcium chloride solution that preserved the construction geometry.

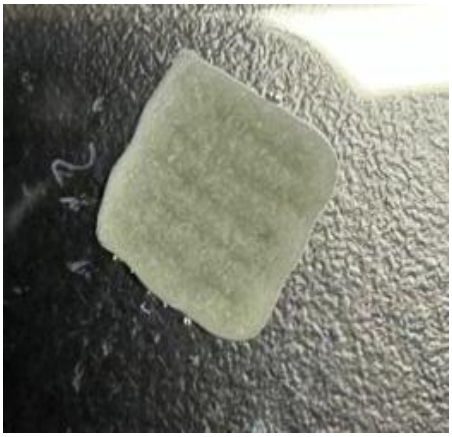

Figure 4. Picture for carbopol scaffold loaded with PCL/PLGA JNPS loaded with DOX and CUR.

In Vitro Drug Release Study

Cumulative Drug Release:

Biphasic release patterns for doxorubicin and curcumin were observed in the 3D-printed structures. A near zero-order release was found over 14 days, with an initial burst of surface-bound drug released in the first 24-48 hours. Such biphasic release assists in building a medicinal effect in no time, on the other hand, it provides an overextended existence of the drug. The PCL phase, in contrast with the PLGA phase, was more hydrophobic (and positively) toluene compatible which resulted in a slow release of Curcumin and doxorubicin released

faster because of its more aqueous solubility. This unique release profile demonstrates that Janus nanoparticles can alter drug-release kinetics, which could potentially be used to tailor treatment regimens (Fig.5).

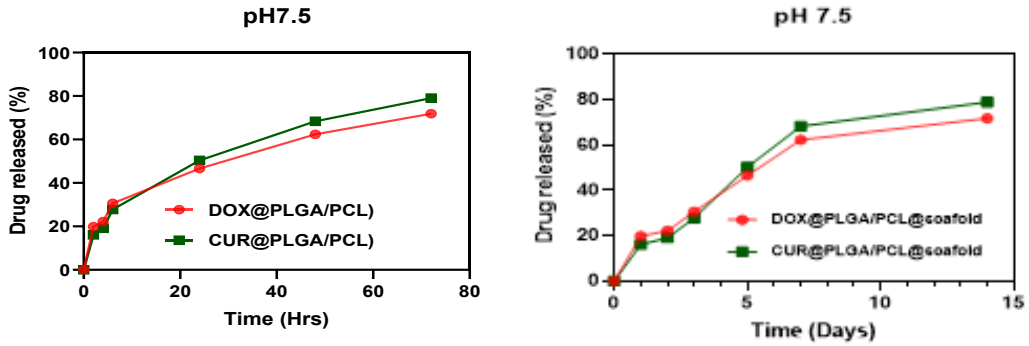

Figure 5. In vitro release of DOX and CUR from PLGA/PCL JNPS and PLGA/PCL JNPS@3d bio-printed scaffold

Conclusion

In the present study, a new multi-drug delivery system using 3D bioprinting was developed simply by mixing PLGA/PCL Janus particles with doxorubicin and curcumin in a Carbopol bioink. The Janus structure of the nanoparticles was able to package and release both hydrophilic drugs (DOX) and hydrophobic drugs (CUR), making it an even more flexible platform for combination therapy. By tuning the bioink's rheological properties for 3D bioprinting, we achieved higher levels of structural integrity and resolution. It was found that nanoparticles did not affect the mechanical properties of the bio-ink, nor the printing results, compared to no-nanoparticle formulations—a finding that supports this technique as an effective method for generating complex 3D structures. The system demonstrated a biphasic release profile, i.e., an initial burst followed by a sustained release in *in-vitro* drug release tests. This profile is key for maintaining therapeutic drug levels throughout a dosing interval. The cytotoxicity study on cancer cells demonstrated an enhanced apoptotic response from the two drug-loaded constructs, indicating that the combination therapy of doxorubicin and curcumin exhibited a synergistic effect. In conclusion, this study offers a blueprint for developing nanoparticles as an ultra-flexible and rapid medication delivery system using cutting-edge nanoparticle technology along with 3D bioprinting. These positive in vitro results provide a foundation for more thoroughly studying the therapeutic efficacy and safety of this approach in subsequent studies using relevant animal models. This method is an applicable strategy for targeted drug delivery and personalized medicine, where it could be extended to different medication combinations or therapeutic fields

Recommendations

The study's conclusions lead to the following suggestions for further investigation and advancement in the area of 3D bio-printed medication delivery systems: Even though the in vitro results are encouraging, more in vivo research is necessary to assess the safety, biodistribution, and therapeutic efficacy of the PLGA/PCL Janus nanoparticles loaded with doxorubicin and curcumin. The goals of these investigations ought to be to ascertain the ideal dosage schedule and comprehend the pharmacokinetics and pharmacodynamics of the dual-drug system in a living being.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

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References:

Alvisi, N., & de Vries, R. (2023). Biomedical applications of solid-binding peptides and proteins. *Materials Today Bio*, *19*, 100580.

Du, J., Wei, Q., & Li, X. (2023). Biocompatibility and drug release properties of 3D printed scaffolds in biomedical applications. *Journal of Biomedical Materials Research Part A*, 111(5), 873-888.

Gupta, S., Zhang, Y., & Kumar, R. (2023). Targeted cancer therapies: Advances and challenges. *Current Oncology*, *30*(3), 1917-1929.

Gupta, P., & Sharma, A. (2023). Nanocurcumin-doxorubicin conjugates: Emerging combination therapy for cancer. *European Journal of Pharmaceutical Sciences*, *180*, 106292.

Kesharwani, P., & Jain, S. (2023). Curcumin and its combination with doxorubicin: A promising approach in cancer therapy. *Biomedicine & Pharmacotherapy*, *162*, 114639.

Li, C., Zhang, Y., Zhou, X., & Xu, Z. (2023). Janus nanoparticles for dual drug delivery in cancer therapy: A review. *Nano Today*, *48*, 101636.

Liu, H., Zhang, L., & Chen, W. (2023). Advances in 3D bioprinted scaffolds for controlled drug delivery in tissue engineering. *International Journal of Molecular Sciences*, *24*(5), 2763.

Rahman, S., Akter, S., & Ahmed, T. (2023). 3D bioprinting of scaffolds for drug delivery applications: Recent advancements and future prospects. *Bioengineering*, *10*(2), 198.

Ranjan, S., Das, B., & Roy, P. (2023). PLGA-based biodegradable nanoparticles: A review of recent advances in drug delivery. *International Journal of Pharmaceutics*, *629*, 122404.

Singh, P., & Sharma, A. (2023). Advances in polycaprolactone-based nanocarriers for cancer drug delivery. *Journal of Controlled Release*, *355*, 298-310.

Tan, K. F., In, L. L. A., & Vijayaraj Kumar, P. (2023). Surface functionalization of gold nanoparticles for targeting the tumor microenvironment to improve antitumor efficiency. *ACS Applied Bio Materials*, *6*(8), 2944-2981.

Wong, K. M., Ooi, A., & Loke, S. Y. (2023). Overcoming chemotherapy resistance in cancer: Current trends and future directions. *Drug Resistance Updates*, *65*, 100896.

Yu, Y., Zhao, H., Shi, C., Fang, Y., Feng, Y., & Zhao, L. (2023). Study on the effect of different fillers on tablet properties of natural plant products: Quality regulation and formulation recommendation. *Journal of Drug Delivery Science and Technology*, *84*, 104444.

Zhang, W., Li, X., & Wang, Y. (2023). Controlled drug release from 3D bioprinted scaffolds for tissue engineering: Recent advances and future directions. *Materials Science and Engineering: C*, *139*, 112543.

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