Hanging Drop Cell Culture Followed By Pulse Laser Assisted High Throughput Intracellular Delivery

IITM Technology Available for Licensing

Problem Statement

- Traditional 2D cell cultures are convenient but **don't accurately represent real-life conditions**, especially in disease models. They **struggle** to mimic patient tissues for drug testing and disease modeling. In 2D cultures, **drug diffusion is limited,** making it **hard to control dosages.**
- 3D spheroids, with their 3rd dimension, **better mimic in-vivo responses** to external factors like physical and chemical signals. Traditional methods for introducing substances into cells, like viruses or chemicals, have issues like **invasiveness and contamination risks.**
- Laser-based methods, like **optoporation**, offer a solution for uniform and localized substance delivery. They are **efficient, selective, reproducible, and don't require physical contact.** Ultrashort laser pulses are particularly effective for **creating tiny pores in cell membranes,** allowing substances to enter while keeping cells healthy.
- To address the above mentioned issues, the present patent is introduced.

Technology

The present patent discloses a **hanging drop cell culture followed by pulse laser assisted high throughput intracellular delivery method,** (Refer FIG 1, 2, 3); the present patent comprising:

- preparing hanging drop consisting of SiHa cells by trypsinizing the cultures cells wherein the supernatant are removed and cell pellets are r-suspended in cell culture medium (DMEM-F12 (Invitrogen) supplemented with 1% MEM Non-Essential Amino Acids (Gibco), 5% fetal bovine serum and 1% penicillin-streptomycin);
- incubating the spheroids with corrugated Gold (Au) nanoparticles to facilitate optoporation wherein the particular corrugated shaped Au-nanoparticles generate high surface plasmonic resonance owing to high electromagnetic field enhancement under exposure of nanosecond pulsed laser wherein cells are exposed at 680nm wavelength, 9 mJ energy for 30 sec;
- and using Propidium Iodide (PI) dye as cargo to be delivered inside spheroid in hanging drop culture wherein after photoporation cells are stained with Calcein dye to test viability of cells.

Technology Category/ Market

Category: Micro & Nano Technologies, Assistive, Test Equipment & Design Manufacturing

Industry: Bio-Micro/Nano, Biomedical Engineering

Applications: Laser assisted optoporation on hanging drop culture, Organoid, Drug delivery, Cellular therapeutics-diagnostics, Cancer treatment

Market: The global 3D cell culture market was **USD 1,700M in 2022** and is estimated to be worth approximately **USD 6,529M,** growing at a **CAGR** of **14.8%** between **2023 & 2032.**

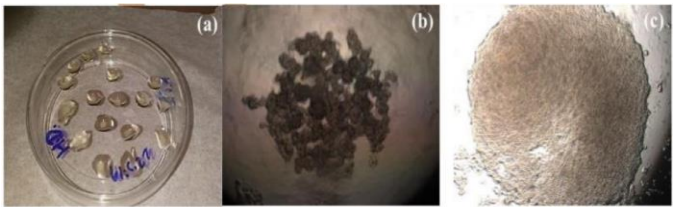
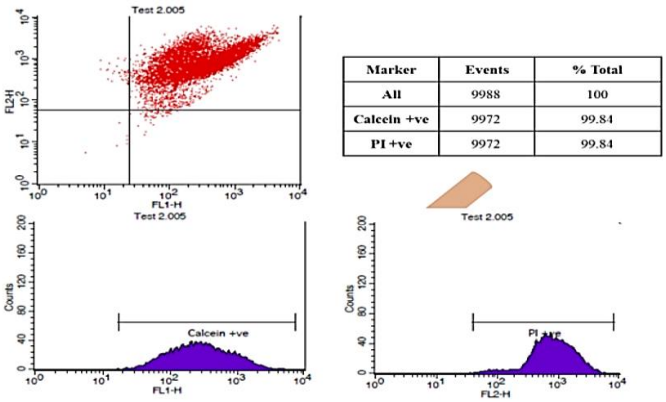


FIG. 1 illustrates graphical images
(a). hanging drop cultures for SiHa cells,
(b). cell spheroid at lower SiHa cell density
(c) higher cell density



Marker	Events	% Total
All	9988	100
Calcein +ve	9972	99.84
PI +ve	9972	99.84

FIG. 2 illustrates graphical representation of flow cytometry analysis to show the optoporation on hanging drop spheroid culture wherein the spheroid shows the almost **similar cell count for PI +ve & Calcein +ve cells, concluding high optoporation efficiency on the spheroid**

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Key Features / Value Proposition

❖ **User Perspective:**

- Biomedical researchers & pharmaceutical scientists looking to **improve drug delivery methods** and develop more **physiologically relevant in vitro models** for drug screening and disease modeling.
- Tissue engineers and regenerative medicine researchers looking to **create artificial tissues and organs for transplantation**. Oncologists and researchers interested in personalized **cancer treatment** approaches and studying the **behavior of cancer cells in 3D environments.**

❖ **Industrial Perspective:**

- Pharmaceutical & biotechnology companies seeking innovative techniques for **drug discovery and development, and personalized medicine applications.**
- Companies in the field of **regenerative medicine seeking efficient methods for producing transplantable tissue/organs.** Companies specializing in **cancer diagnostics, treatment,** & **precision medicine, aiming to optimize therapies** based on patient-specific responses.

❖ **Technological Perspective:**

- Hanging drop cultures & laser-assisted optoporation offer potential advancements in the creation of **functional 3D tissues for regenerative medicine applications.**
- Laser-assisted optoporation in hanging drop cultures provides a platform for **testing and optimizing personalized cancer treatments before clinical application**. Advances in laser technology and nanoparticle-based delivery systems are driving innovation in drug delivery methods, offering **precise and controlled intracellular delivery in 3D cell cultures.**

Intellectual Property

IITM IDF Number: 2044
Application Number: 202041033827

TRL (Technology Readiness Level)

TRL – 3; Proof of Concept

200

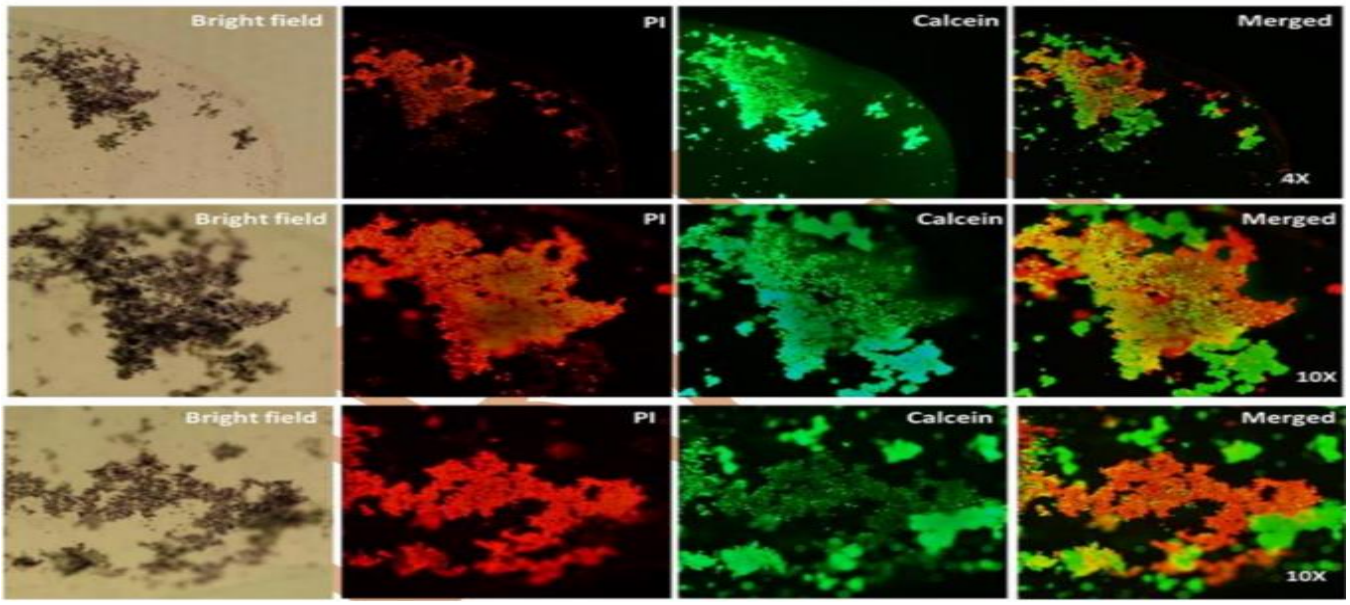

FIG. 3 illustrates optical images **200** showing the laser assisted **Au nanoparticle** mediated optoporation of **PI dye 219 in SiHa cells**, stained with **Calcein 220** to show viability of cells after optoporation wherein the **cells 230 represent PI delivered live cells**

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