



## Modulated substrate-controlled plasmid transfer to cell monolayer

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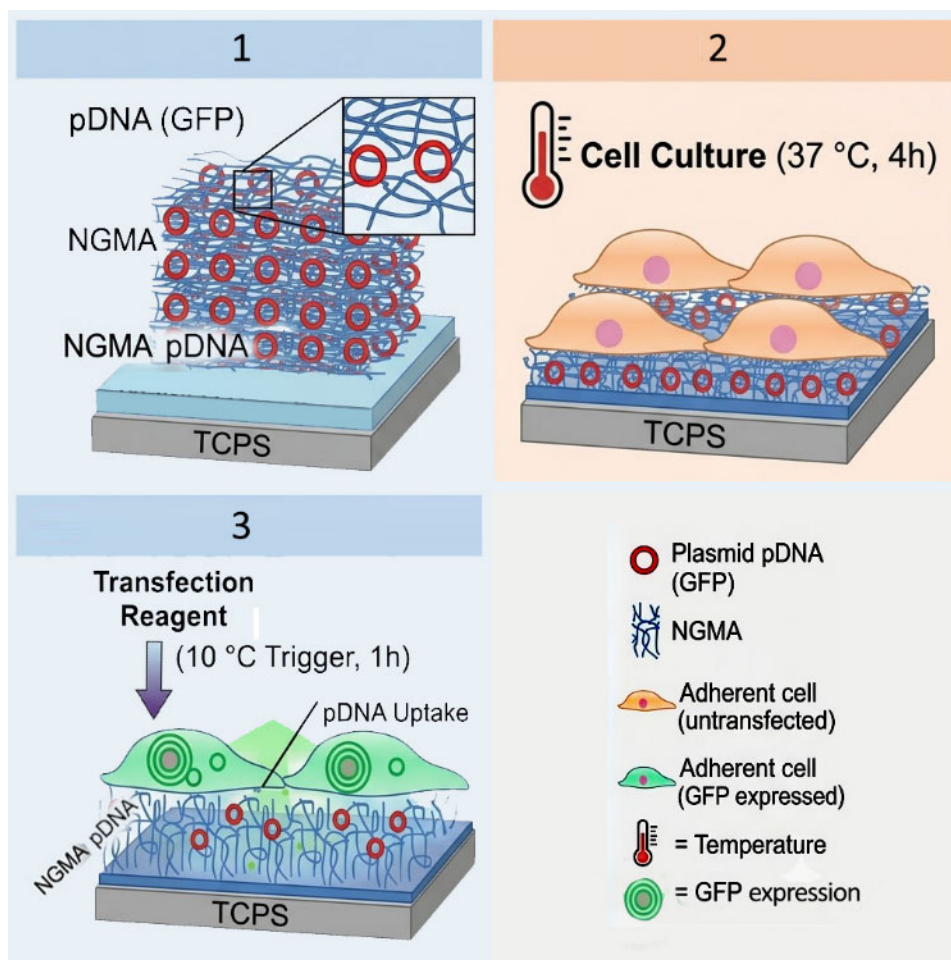
Efficient and controlled gene delivery remains a critical requirement in biomedical research, particularly for applications in tissue engineering, gene therapy and cell-based assays. Conventional non-viral gene delivery methods often face limitations such as low transfection efficiency, lack of spatial control and cytotoxicity. Hence there is need for innovative platforms that enable precise, efficient and localized gene transfer. In this study, in-house developed Poly(N-isopropylacrylamide)-Co-Glycidyl Methacrylate (NGMA) polymer has been evaluated as a non-viral gene transfer platform by transferring plasmid DNA encoded with Green Fluorescent Protein (GFP). The NGMA-plasmid complexes were coated onto tissue culture polystyrene surfaces and L-929 cells were cultured on these surfaces. Gene transfer was induced through temperature modulation in the presence of a transfection reagent, enabling intracellular uptake and functional expression of GFP. The delivery of plasmids into the cell monolayer was evaluated in different conditions. Results demonstrated rapid and efficient cellular internalization of plasmid DNA, with distinct GFP expression observed only on polymer-coated regions, confirming the spatial and temporal control of this solid-state gene delivery system. This novel approach merges non-viral gene delivery with substrate-associated cell culture, providing a versatile platform for generating genetically modified cell monolayers.

**Keywords:** GFP, NGMA, Plasmid, Thermoresponsive

Gene or DNA delivery is a complex process of tightly involved steps condensation or packing of DNA, transporting of DNA in to the cells, facilitates its release and expression of gene and subsequent synthesis of protein of interest. Though a number of vectors for gene therapy has been studied so far the delivery of negatively charged DNA through negatively charged plasma membrane also not feasible. Successful formulation of a gene delivery system requires thorough understanding of the mechanism between tracking of the gene and target cells. DNA can be inserted into cells using a range of techniques including chemical, electrical, lipid/protein-based and viral methods<sup>1,2</sup>. Each of the methods has its own advantages and disadvantages. A very common vector used to insert DNA into cells is plasmid which is a small circular DNA to which the gene of interest is inserted along with genes for selecting the transfected cells. Green Fluorescent Protein (GFP) is a well-established marker used in plasmids to track the gene delivery and protein expression<sup>3</sup>. Generally, plasmids are provided in

medium reagents that promotes transfection. This solution-based method has challenges in the degradation of DNA and complex formation in liquid state gene transfer. Synthetic polymers have become an alternative to viral vectors in gene delivery<sup>4-6</sup>. Stimuli responsive polymeric hydrogel has been reported to stabilize plasmids and augment the transfection process<sup>7,8</sup>. Thermoresponsive polymers or smart polymers are class of synthetic materials capable of change in the property based on the change in temperature render them as a good agent for delivering drugs, biomolecules and gene delivery to the cells or tissues<sup>9,10</sup>. These materials have advantage over viral vector due to low cost and ease of production. Smart polymers have major attraction in the field of tissue engineering applications as they are capable of inserting gene in to cells and there by treating diseases at the molecular level. There are many studies so far reporting the use of thermoresponsive polymer in gene delivery applications<sup>2,10-12</sup>. In a study based on poly(ethyleneimine) (PEI) with grafted neutral responsive poly(N-isopropylacrylamide) (PNIPAM) the DNA was condensed in to particles with architecture varying with LCST of graft polymer reported<sup>13</sup>.

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Graphical abstract

Another study also detailed using chitosan grafted (PNIPAm) as a gene delivery vector<sup>14</sup>. The compressed hydrophobic globule protects DNA from nucleases prior to and during intracellular transport. Inside cell it enhances to a hydrophilic structure and there by release DNA causing transfection.

In this study we propose to provide plasmid entrapped in a thermoresponsive polymer matrix to cells that are cultured on it. The in-house developed Poly(N-isopropylacrylamide)-Co-Glycidyl Methacrylate (NGMA) polymer expresses thermally induced phase transition around a Lower Critical Solution Temperature at 28°C that has been proved to be useful in cell sheet engineering<sup>15</sup>. The NGMA Co-polymer based 'solid state' transfection process involves forming polymer-plasmid complex solution, coating tissue culture plates with these complexes, culturing cells on the coated surfaces and study the gene transfer and expression in the presence of transfection reagent and low temperature treatment.

## Materials and Methods

### Materials

NIPPAm was purchased from Acros Organics, India. GMA (Guanidyl methacrylate), 2,2 0-azobis(isobutyronitrile) (AIBN) and tetrahydrofuran (THF), isopropanol were obtained from Merck Chemicals, India. Polystyrene culture dishes for coating polymer were purchased from Nunc,US. Cell culture reagents, Minimum Essential Medium (MEM), Glutamine, Sodium bicarbonate Trypsin, Neutral red, Penicillin, and Streptomycin, was purchased from Sigma, India. Foetal Bovine Serum (GIBCO, USA), pEYFP N1 plasmid from Clontech and Cell Transfection kit Transit 2020 (Mirus).

### Synthesis of thermo responsive polymer

The NGMA copolymer was prepared by previously reported method<sup>15</sup>. Briefly, N-isopropyl acrylamide and Glycidyl Methacrylate was copolymerized at 60°C under a blanket of nitrogen gas and AIBN as

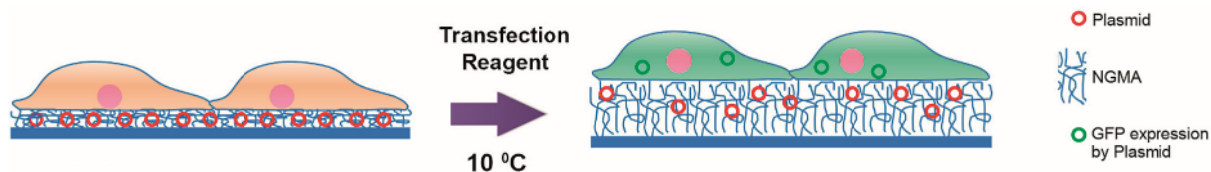


Fig. 1 — Diagrammatic representation of solid state gene delivery model

initiator. The polymer was precipitated using cold water and dissolved in AIBN for three times to remove monomers of polymer. The precipitate was dried in hot air oven at 60°C overnight. The final product was dissolved isopropanol for cell culture studies. Thermoresponsive efficiency was studied by lowering the temperature of polymer to below LCST (lower critical solution temperature) of the polymer. The LCST of NGMA co polymer was found to be below 28°C. The phase transition property of the polymer was explored to deliver plasmid inside cells.

**Preparation of gene transfer plates**

Polymer-Plasmid complex was prepared by mixing 400 and 4000 µg/mL NGMA in isopropanol with 6 µL of plasmid of GFP tagged plasmid. The complex was then coated in 35mm cell culture dish and dried at 50°C overnight. The plates were washed with ice cold serum free MEM thrice to remove unbounded polymer.

**Efficacy of transfection reagent**

To ensure the efficacy of plasmid and the transfection reagent, L-929 cells cultured on 35 mm tissue culture polystyrene dishes were transfected based on conventional method. Plasmid DNA was diluted in serum-free medium and Lipofectamine reagent was separately diluted in the same medium according to the manufacturer’s instructions. The complexes were then added dropwise to the cells and incubated for 24 h at standard culture conditions (37°C, 5% CO<sub>2</sub>) for 24 h. After incubation, GFP expression was assessed using fluorescence microscopy to confirm successful plasmid delivery and gene expression

**Cell culture on NGMA-Plasmid dishes**

L929 mouse fibroblast cells purchased from American Type Culture Collections (ATCC, USA) was cultured in MEM supplemented with 300mM glutamine, 7.5% sodium bicarbonate 10% FBS, 100 U/mL of penicillin and 100 µg/mL streptomycin and maintained in a CO<sub>2</sub> incubator. Cell monolayer was treated with Trypsin (0.2%) -EDTA (0.02%) solution

Table 1 — Experiment groups to evaluate the solid state gene transfer

Method	Temperature treatment	Medium
1	37°C	MEM
2	10°C, 20 min	MEM
3	37°C	MEM + Transfection reagent
4	10°C, 20 min	MEM + Transfection reagent

to obtain as suspension in culture medium. Cells were seeded on NGMA plates coated with polymer-plasmid complex prepared using 400 and 4000 µg/mL concentration of polymer. The cells on NGMA-plasmid plates were maintained in a CO<sub>2</sub> incubator.

**Solid State Thermo-modulated gene transfer**

Cells cultured on NGMA-Plasmid surface were allowed to form monolayer. The temperature was brought down to 10°C and commercially available transfection reagent was added to culture medium. Outline of the work is diagrammatically represented in (Fig. 1).

**Cell culture and transfection experiments**

The solid-state gene transfection plate was prepared by coating the tissue culture dishes with NGMA-plasmid complex formulated using a 400 µg/mL NGMA solution. L929 Mouse fibroblast cells were sub cultured using trypsin and seeded to NGMA - Plasmid plate at a cell concentration of 1 × 10<sup>4</sup> cells/cm<sup>2</sup>. Cells cultured on normal TCPS were taken as control. The experiment was designed to overrule the chances of gene transfer by any means other than by temperature modulation (Table 1) Parallel control cells were transfected with plasmid by conventional solution method.

The gene transfer was confirmed by observing the fluorescence due to expression of GFP inside the transfected cells under an inverted fluorescence microscope (Leica, DMI6000) using green filter (I3). After 1h incubation, culture medium from all cultures was replaced and incubated in CO<sub>2</sub> incubator for 24 h to verify any deleterious effect on cells.

**Confirmation of localized gene delivery**

To confirm the efficiency in gene transfer at localized area of the same culture, the NGMA-

plasmid was coated in pattern. A thin circular area in the 35 mm tissue culture dish was coated with NGMA-plasmid. L-929 cells were cultured as described before.

#### Cell viability after transfection

Cells exposed to the successful gene transfer using NGMA-plasmid dishes were cultured for 24 h and subjected to Neutral Red staining. 0.5% neutral red solution in PBS was added to cell monolayer on NGMA-plasmid dishes and incubated for 20 min at room temperature ( $25\pm 3^\circ\text{C}$ ). The cells were then observed under inverted light microscope (Motic, China)

#### Results and Discussion

Gene therapy is fundamentally constrained by the difficulty of delivering nucleic acids into target cells without losing the efficiency of transfection while minimizing cytotoxicity and preserving gene expression<sup>16</sup>. Among non-viral vectors, thermo responsive polymers like PNIPAM or PNIPAM based polymers have attracted significant interest for drug and gene delivery due to their reversible phase transition behavior in response to temperature changes<sup>4,7,17</sup>. PNIPAM exhibits a lower critical solution temperature (LCST) of approximately  $32^\circ\text{C}$  is very close to the body temperature ( $37^\circ\text{C}$ ), enabling temperature-controlled modulation of polymer–biomolecule interactions<sup>18</sup>. Polymeric gene delivery system typically involves sequential steps such as electrostatic condensation of plasmid DNA with the polymer to form a polyplex, incubation of the polyplex with cells to facilitate cellular uptake *via* endocytosis and intracellular trafficking and expression of the gene of interest.

A major limitation arises while using existing systems is the gene uptake by cells and subsequent

removal of polyplexes from the cell surface<sup>19</sup>. This limitation ultimately can compromise cell viability and transfection efficiency. The success rate of transfection depends on the delivery of gene of interest in to the cells without rendering any damage to the cells. Interestingly, thermoresponsive polymers provide a unique strategy to address this limitation, as changes in temperature below the LCST induce polymer hydration and conformational expansion, enabling controlled release of the genetic payload without harsh chemical or mechanical stimuli. The most successful application of the PNIPAM was reported as a substratum for the culture and development of cell sheet for tissue engineering applications<sup>12</sup>. A co-polymer formulation known as Poly(N-isopropyl acrylamide)-Co-Glycidyl Methacrylate or NGMA has been reported as a temperature-responsive cell culture substrate, allowing nonenzymatic harvesting of intact cell sheets for tissue engineering applications. Building on this concept, NGMA has been used as a ‘solid state’ gene delivery platform, designed to achieve controlled plasmid DNA release directly to cell monolayer with simple change in temperature within short duration. This design enables selective release and cellular internalization of plasmid DNA while minimizing polymer-associated cytotoxicity, thereby addressing a key challenge in thermoresponsive polymer-mediated gene delivery systems.

#### Efficacy of transfection reagent

The efficiency of cell transfection reagent also confirmed using conventional solution based where the plasmid is complexed with transfection reagent and incubated for 24 h with cells on TCPS. GFP expression observed after 24 h of incubation with plasmid complex as shown in (Fig. 2).

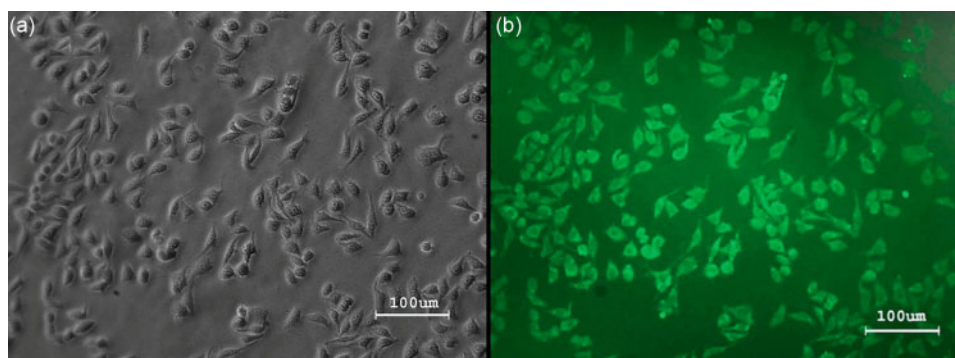


Fig. 2 — Lipofectamine-mediated transfection of plasmid DNA in L-929 cells cultured on TCPS. (a) Cells incubated plasmid with gene transfection reagent; and (b) GFP expression in cells confirms the efficiency of the conventional solution-based transfection method

### Cell culture on NGMA-plasmid plates

The concentration of NGMA plays a crucial role in the release and uptake of plasmid<sup>20</sup>. The thin layer of approximately  $<10\ \mu$  thickness is more efficient in maintaining cell morphology. A thick layer is more hydrophilic than the thin and there will be difficulty in cell adhesion and proliferation<sup>12</sup>. The concentration of polymer is also a critical factor in thermoresponsiveness, when the polymer is used for coating cell culture surface and plasmid entrapment. Higher concentration brings more polymer chains which affect the release of GFP-plasmid from the polymer. NGMA at higher concentration (4000  $\mu$ g) showed distinct white precipitate at 37°C and this induced cytotoxicity of cells by polymer and the proliferation was less (Fig. 3). Hence the lower concentration of 400  $\mu$ g was taken for transfection studies. Introduction of decreased toxicity and high transfection efficiency is considered as one of the major criteria in choosing synthetic polymers as gene carriers<sup>2</sup>. Since the NGMA is non toxic and stable at room temperature as the cells are growing and proliferating on coated surfaces. The highest concentration of Polymer causes a coiled structure which will prevent the release of plasmid during change in temperature<sup>21</sup>. The low concentration of NGMA is also helpful in forming polyplexes with less penetrating network which in turn enhance the rate of transfection<sup>7</sup>.

### Gene transfection experiment

The gene transfection experiments were carried out by analyzing various factors affecting the delivery and expression of GFP tagged plasmid inside cells. All critical factors were investigated to rule out the effectiveness of proposed method of gene delivery systems. Solution based gene delivery system is the most widely accepted protocol for gene delivery while

using synthetic carriers<sup>7</sup>. Before expressing the desired protein the synthetic DNA carriers have to navigate series of obstacles both intracellular and extracellular<sup>22</sup>. Our proposed solid state gene delivery system outweighs the major challenge in solution based gene carriers as the cells are directly cultured on polymer-plasmid coated surfaces and which in turn enhances the efficiency of transfection. Since the success of newly proposed system depends on various factors like, temperature, transfection reagent and time of incubation each and every parameter were analyzed to avoid unintended interference of factors affecting the efficacy of transfection.

### *Gene transfer at normal culture condition (37°C) without transfection reagents*

At normal culture condition the polymer DNA complex were compacted above LCST and that may be the reason behind no specific release and uptake of plasmid by cells<sup>7</sup>. The cells were attached and proliferated in coated surface as shown in (Fig. 4). Since there was no discernable difference in Plasmid expression observed at 37°C, the experiment substantiate temperature as one of the major factor in thermos-response gene delivery experiments. This also supports the pervious findings of Sang-oh Han *et al*<sup>23</sup>, where the transfection efficiency of a terpolymer, is high at low temperature due to the formation/dissociation control of thermosensitive polymer. Temperature plays crucial role in the release of plasmid from polymeric network of copolymer of PNIPAm and GMA again substantiate by the R.Yoshida<sup>24</sup>. They have reported that the fast response to temperature stimuli, the drug can be released from the hydrogel quickly and act as an on-off switching release system. The Terpolymer/DNA complex was easily being dissociated below the LCST, while above the LCST, these complexes were

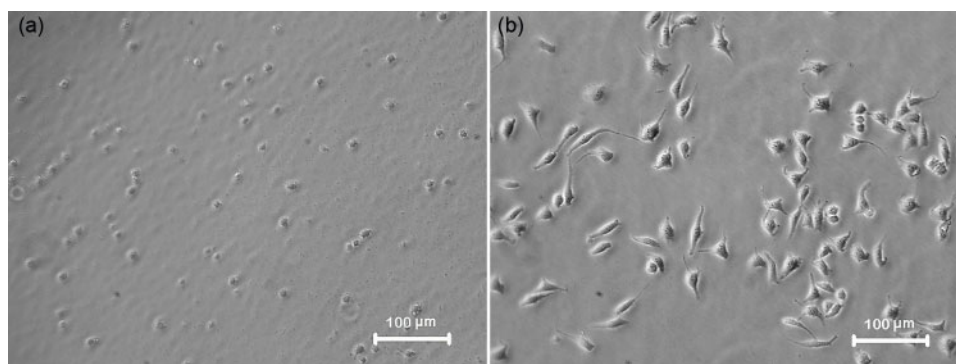


Fig. 3 — L-929 cells cultured on plasmid entrapped NGMA at different polymer concentrations (a) 4000  $\mu$ g/mL; and (b) 400  $\mu$ g/mL. At higher concentration, the NGMA-Plasmid showed cytotoxicity and at low concentrations non-cytotoxic

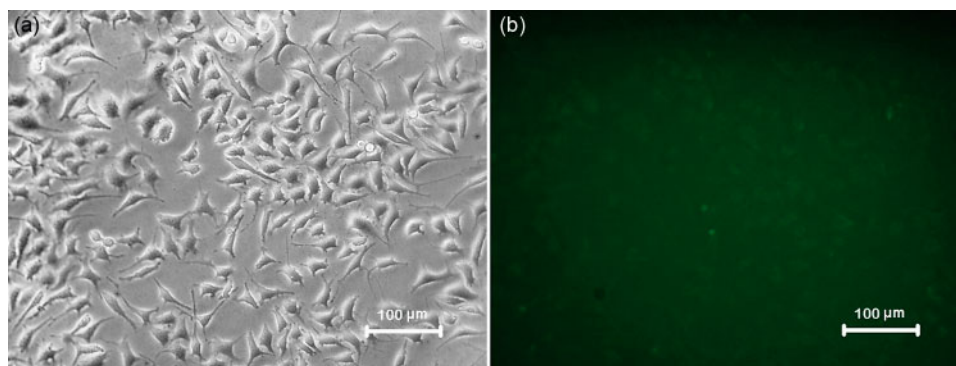


Fig. 4 — (a) L-929 cells cultured on NGMA-Plasmid surface; and (b) Absence of GFP expression by cells shows no occurrence of gene delivery during normal culture conditions

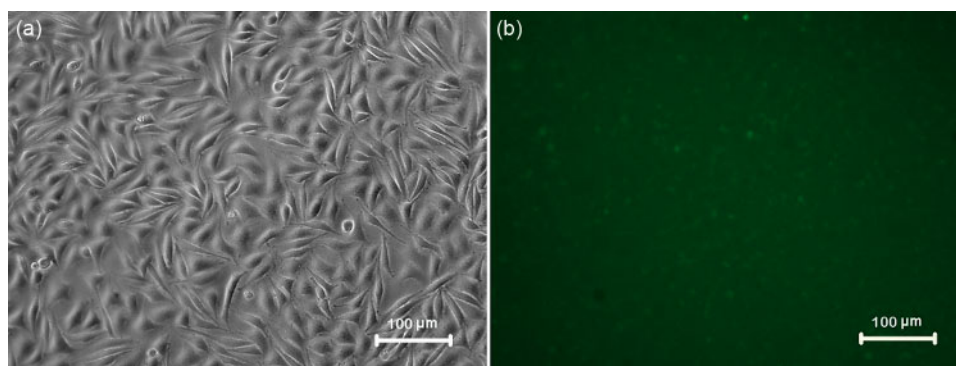


Fig. 5 — L-929 cells on NGMA-Plasmid surface. (a) Cells incubated at 10°C for 20 min in normal culture medium; and (b) Absence of GFP expression confirms absence of gene transfer

formed by additional hydrophobic interaction due to thermo-responsive copolymer aggregation. The results demonstrated that L-929 cells cultured on the NGMA-Plasmid surface under normal culture conditions did not express GFP, indicating negligible gene transfer when plasmid DNA is merely presented on the cell culture surface without additional facilitating factors.

#### *Gene transfer at LCST without transfection reagents*

Temperature treatment alone could not able to release plasmid from the polymer as there was no gene expression by method 2 (Fig. 5). Here the cells were treated to below LCST of polymer but no significant expression of plasmid was observed when transfection reagent was not added to the culture system. This confirms the pivotal role of transfection reagent in solid state gene delivery. Mirus 2020 low toxic transfection reagent was used to carry plasmid DNA to the cytoplasm of the target cells. The results suggest that absence of transfection reagent even if the temperature of the polymer is lowered to below LCST, the plasmid could not be internalized. The cells exposed to a transient low-temperature (below

LCST) did not express GFP, underscoring that temperature modulation alone is insufficient to trigger internalization of plasmid DNA from a solid interface. Thus the above observations evidenced by the absence of GFP gene expression under standard cell culture conditions and short duration temperature stimuli does not inherently confer efficient cellular. This also confirms that the NGMA-plasmid plate is behaving like normal tissue culture grade surface under standard conditions. This is consistent with studies showing that substrate-immobilized DNA, without additional facilitating vectors or mechanical/physicochemical triggers, is poorly internalized by adherent cells because the critical steps of plasmid detachment and membrane translocation are rate-limiting barriers<sup>25</sup>.

#### *Gene transfer at normal temperature (37°C) with transfection reagent*

The possibility of plasmid internalization as per standard lipofectamine based gene transfer protocol was also studied. It was performed to show whether the plasmid in NGMA-plasmid complex coatings or free plasmids from the coated surface are available to

give false positive results. Conventional transfection reagents (*e.g.*, lipofectamine) operate by complexing with plasmid DNA in the medium to enable endocytic uptake into the cells<sup>8</sup>. NGMA-plasmid system is same as conventional system of gene delivery by complexing released plasmid with transfection agent. The results showed that cells cultured with a conventional transfection reagent did not show fluorescence of GFP gene expression, confirming that soluble transfection complexes do not carry free plasmids on its surface to be delivered into cells (Fig. 6). The lack of GFP expression in Figure 6 in the presence of transfection reagent supports the notion that plasmid DNA was not accessible to the cells.

#### *Gene transfer below LCST with transfection reagents*

The conventional procedure of gene transfection involves complexing the non-viral carrier with a plasmid DNA and then adding it to a cell culture medium. This requires incubation of the target cells with culture medium for 24 h prior to transfection.

Besides serum cannot be used for transfection experiment as it affects the process of DNA-carrier complex thereby suppressing the efficacy of transfection. Studies reported that Reverse transfection is found to be more successful in delivering plasmid DNA than conventional solution based methods<sup>26</sup>. One of the desirable criteria for using thermoresponsive polymers is it should not interfere with transfection efficiency of plasmid. The formation of polyplex neither affects the transfection efficiency nor the constant expression potential of plasmid.

In this study temperature stimuli and presence of transfection reagents showed that the plasmid gene (GFP tag) was expressed inside the cells (Fig. 7). This demonstrate that during cold incubation plasmid was released from the NGMA surface, and in the presence of transfection reagent, the released plasmid was internalized and expressed by the cells. The expression of plasmid inside cells again confirms the efficiency of solid-state transfer over other

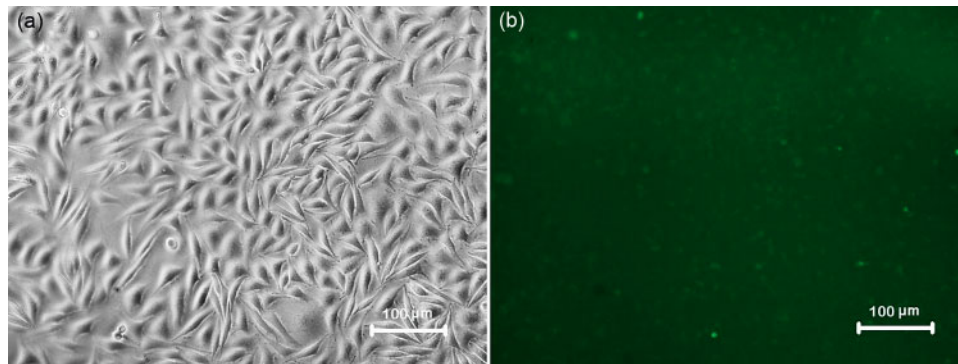


Fig. 6 — Assessment of plasmid internalization and potential false-positive GFP expression using a conventional lipofectamine-based transfection control. L-929 cells on NGMA-Plasmid surface. (a) Cells incubated at 37°C in medium supplemented with gene transfection reagent; and (b) There was no detectable GFP fluorescence indicating absence of free plasmid DNA to the cells

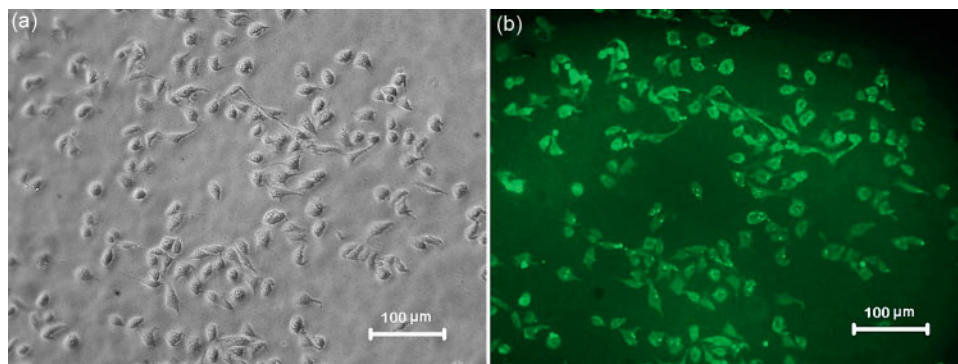


Fig. 7 — Temperature-triggered plasmid release and intracellular expression of GFP in the presence of a transfection reagent. L-929 cells on NGMA-Plasmid surface. (a) Cells incubated at 10°C in medium supplemented with gene transfection reagent observed under phase contrast microscope. Cold incubation induced the release of plasmid DNA from the NGMA surface, and subsequent exposure to a conventional transfection reagent enabled cellular internalization and expression of the GFP-tagged plasmid; and (b) GFP expression in cells confirms efficient gene transfer

conventionally available system of gene delivery. The plasmid showed distinct green fluorescence by expressing functionality of plasmid inside the cell. This mechanism aligns both with conventional non-viral transfer behavior once DNA is soluble and with substrate-associated delivery logic where the temporal control of DNA availability at the cell–material interface is critical for successful gene transfer.

#### Confirmation of localized gene delivery

The effectiveness of the solid-state gene transfer was further evaluated by coating the middle portion of the TCPS with NGMA–Plasmid complex and other portion was left without coating. The experiment was repeated with lowering the temperature below LCST with transfection reagents and it again substantiate the proposed method of solid-state gene delivery system as shown in (Fig. 8). Distinct green fluorescence confirms functional gene expression within cells, demonstrating that the plasmid became soluble, was

effectively internalized only in the portions coated with NGMA–plasmid. The uncoated area in the same dish did not show fluorescence within 20 min of incubation. These results validate the efficiency of the NGMA-based solid-state gene transfer system temporally controlled DNA availability at the cell–material interface.

#### Cell viability after transfection

Cell viability by neutral red staining after transfection showed the presence of viable cells indicating the functionality not affected by transfection (Fig. 9). The cells showed similar pattern of neutral red staining with control TCPS indicates the viability was not affected by the change in temperature and transfection reagent.

#### Advantages of solid-state gene transfer

In conventional gene transfer (soluble delivery) DNA or DNA complexes are presented to cells in the

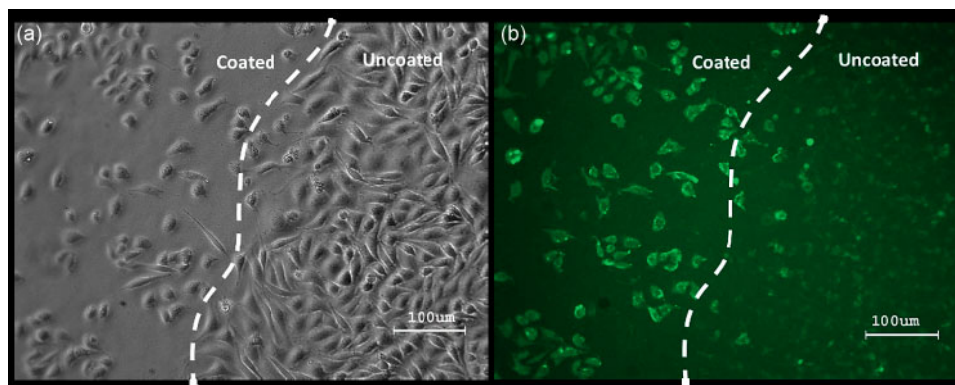


Fig. 8 — Spatially localized solid-state gene transfer from NGMA–plasmid–coated TCPS surfaces. (a) The central region of the TCPS dish was coated with NGMA–plasmid complexes, while the surrounding area remained uncoated. L-929 cells on NGMA–Plasmid coated and uncoated surface incubated at 10°C in medium supplemented with gene transfection reagent where GFP fluorescence was observed exclusively in cells on the NGMA–plasmid–coated region; and (b) GFP expression in cells at NGMA–plasmid–coated region of the culture dish confirms effective localized plasmid release, cellular internalization and functional gene expression directly to the cells. No fluorescence was detected in the uncoated regions

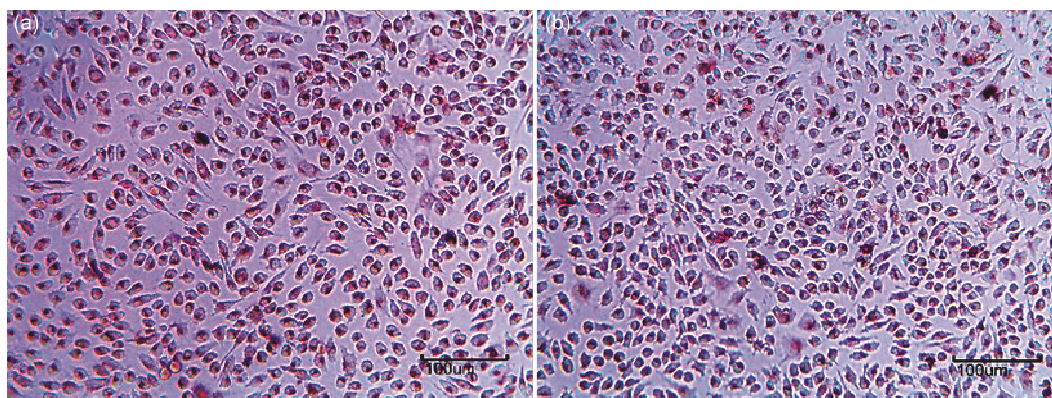


Fig. 9 — L-929 cells on (a) After 48 h of transfection NGMA-Plasmid surface; and (b) control TCPS

form of suspension. A chemical transfection reagent (lipofection, polyfection), physical techniques (electroporation, microinjection, magnetofection) or viral vectors are used for gene transfer. Non-viral chemical methods rely on cationic lipids or polymers to condense plasmid DNA into complexes that can interact with negatively charged cell membranes and be internalized *via* endocytosis. These methods require plasmid DNA to be in solution and are widely used for *in vitro* applications due to their relative simplicity and controllability. However, this method faces several constraints, such as inefficient mass transport to adherent cells, diffusion-mediated loss of plasmid DNA and nucleic acid degradation within culture media, that reduces the transfection efficiency in certain cell types. Additionally, lipid-based reagents such as Lipofectamine typically require extended incubation periods to achieve sufficient uptake, which can disrupt cellular membranes, induce cytotoxicity. These combined limitations have driven interest in substrate-mediated or solid-phase gene delivery strategies, which aim to improve transfection outcomes by immobilizing DNA at the cell–material interface, thereby minimizing diffusional losses and reducing reliance on long-duration, cytotoxic lipid exposure. The Solid-state gene transfer (substrate-mediated delivery) involves immobilizing DNA (or DNA complexes) on a culture surface, so that cells contacting the surface experience high local concentrations of DNA<sup>27</sup>. Reverse transfection is one such approach where DNA complexes are pre-adsorbed onto the culture substrate before cell seeding, which enhances local DNA availability<sup>5</sup>. This strategy has been shown to increase transgene expression and the number of transfected cells compared with soluble bolus delivery with improved cell viability.

The results presented in this study confirms that the NGMA incorporated with plasmids can be a good platform for transfection of cell monolayer. This method is in line with the solid state gene transfer reported before<sup>26</sup>. The ‘solid state’ gene delivery platform was evaluated for successful cellular internalization and subsequent transgene expression was confirmed under *in vitro* conditions. As this study was limited to cell culture studies, clinical and preclinical parameters related to safety and efficacy were not assessed. Hence, the efficacy should be validated through functional outcome measures, biomarker analyses and durability of gene expression.

Those studies will be the future steps starting from preclinical evaluation of this solid-state gene transfer system, for generating comprehensive data required for the translation to clinical applications.

### Conclusion

The thermoresponsive polymer based gene delivery was developed and evaluated *in vitro* using L-929 cells using plasmid. The plasmid expressing green fluorescent protein was incorporated within thermoresponsive NGMA and delivered into L-929 cells by temperature modulation. NGMA-plasmid substrate will also have an advantage of retrieval of genetically modified cells from its surface. Preliminary experiment reveals that NGMA is capable of forming complex with plasmid DNA without affecting functionality. The results suggest that plasmid DNA can be coiled in polymer network which can be effectively taken up by cells within a short duration of time without affecting the functionality. More studies require to fine tune the duration of transfection with different cells. Future investigations should therefore extend this platform to gene-specific and disease-relevant models, followed by *in-vivo* studies to evaluate critical safety endpoints such as toxicity, immune responses, biodistribution, and long-term risks including insertional mutagenesis.

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### Conflict of interest

All authors declare no conflict of interest.

### References

- 1 Keles E, Y Song, D Du, WJ Dong & Y Lin, Recent progress in nanomaterials for gene delivery applications. *Biomaterials Science*, 4 (2016).
- 2 Chen CK, Huang PK, Law WC, Chu CH, Chen NT & L LW, Biodegradable Polymers for Gene-Delivery Applications. *Int J Nanomedicine*, 15 (2020) 2131.
- 3 Tombolini R, Unge A, Davey ME, FJ de Bruijn & Jansson JK, Flow cytometric and microscopic analysis of GFP-tagged *Pseudomonas fluorescens* bacteria. *FEMS Microbiol Ecol*, 22 (1997) 17.
- 4 Nakanishi M, Eguchi A, Akuta T, Nagoshi E, Fujita S, Okabe J, Senda T & Hasegawa M, Basic peptides as functional components of non-viral gene transfer vehicles. *Curr Protein Pept Sci*, 4 (2003) 141.
- 5 Pannier AK & Shea LD, Controlled release systems for DNA delivery. *Mol Ther*, 10 (2004) 19.

- 6 Ravi Kumar M, Hellermann G, Lockey RF & Mohapatra SS, Nanoparticle-mediated gene delivery: state of the art. *Expert Opin. Biol. Ther.*, 4 (2004) 1213.
- 7 Kurisawa M, Yokoyama M & Okano T, Gene expression control by temperature with thermo-responsive polymeric gene carriers. *J Control Release*, 69 (2000) 127.
- 8 Godbey WT, Mikos AG, Recent progress in gene delivery using non-viral transfer complexes. *J Control Release*, 72 (2001) 115.
- 9 Shimizu K, Fujita H & Nagamori E, Oxygen plasma-treated thermoresponsive polymer surfaces for cell sheet engineering. *Biotechnol Bioeng*, 106 (2010) 303.
- 10 Twaites BR, de las Heras Alarcón C, Lavigne M, Saulnier A, Pennadam SS, Cunliffe D, Górecki DC & Alexander C, Thermoresponsive polymers as gene delivery vectors: Cell viability, DNA transport and transfection studies. *J Control Release*, 108 (2005) 472.
- 11 Calejo M, Sande S & Nyström B, Thermoresponsive polymers as gene and drug delivery vectors: architecture and mechanism of action. *Expert Opin Drug Deliv*, 10 (2013) 1669.
- 12 Nagase K, Yamato M, Kanazawa H & Okano T, Poly(N-isopropylacrylamide)-based thermoresponsive surfaces provide new types of biomedical applications. *Biomaterials*, 153 (2018) 27.
- 13 Lavigne MD, Pennadam SS, Ellis J, Yates LL, Alexander C & Górecki DC., Enhanced gene expression through temperature profile-induced variations in molecular architecture of thermoresponsive polymer vectors. *J Gene Med*, 9 (2007) 44.
- 14 Mao Z, Ma L, Yan J, Yan M, Gao C & S. J., The gene transfection efficiency of thermoresponsive N,N,N-trimethyl chitosan chloride-g-poly(N-isopropylacrylamide) copolymer. *Biomaterials*, 28 (2007) 4488.
- 15 Joseph N, Prasad T, Raj V, Anil Kumar PR, Sreenivasan K & Kumary TV, A Cytocompatible Poly(N-isopropylacrylamide-co-glycidylmethacrylate) Coated Surface as New Substrate for Corneal Tissue Engineering. *J Bioact Compat Polym*, 25 (2010) 58.
- 16 Mintzer MA & Simanek EE, Nonviral vectors for gene delivery. *Chemical reviews*, 109 (2009) 259.
- 17 Akimoto J, Nakayama M & Okano T, Temperature-responsive polymeric micelles for optimizing drug targeting to solid tumors. *J Control Release*, 193 (2014) 2.
- 18 Heskins M & Guillet JE, Solution Properties of Poly(N-isopropylacrylamide). *J Macromol Sci A Chem*, 2 (1968) 1441.
- 19 Vaughan EE, DeGiulio JV & Dean DA, Intracellular trafficking of plasmids for gene therapy: mechanisms of cytoplasmic movement and nuclear import. *Curr. Gene Ther*, 6 (2006) 671.
- 20 Akiyama Y, Kikuchi A, Yamato M & Okano T, Ultrathin Poly(N-isopropylacrylamide) Grafted Layer on Polystyrene Surfaces for Cell Adhesion/Detachment Control. *Langmuir*, 20 (2004) 5506.
- 21 Yokoyama M, Gene delivery using temperature-responsive polymeric carriers. *Drug Discov Today*, 7 (2002) 426.
- 22 Zanta MA, Belguise-Valladier P & Behr JP, Gene delivery: a single nuclear localization signal peptide is sufficient to carry DNA to the cell nucleus. *PNAS*, 96 (1999) 91.
- 23 Han So, Mahato RI, Sung YK & Kim SW, Development of Biomaterials for Gene Therapy. *Mol Ther*, 2 (2000) 302.
- 24 Yoshida R, Kaneko Y, Sakai K, Okano T, Sakurai Y, Bae YH & Kim SW, Positive thermosensitive pulsatile drug release using negative thermosensitive hydrogels. *J Control Release*, 32 (1994) 97.
- 25 Bengali Z, Pannier AK, Segura T, Anderson BC, Jang JH, Mustoe TA & Shea LD, Gene delivery through cell culture substrate adsorbed DNA complexes. *Biotechnol Bioeng*, 90 (2005) 290.
- 26 Bielinska A, Yen A, Wu H, Zahos K, Sun R, Weiner N, Baker JJ & Roessler B, Application of membrane-based dendrimer/DNA complexes for solid phase transfection *in vitro* and *in vivo*. *Biomaterials*, 21 (2000) 877.
- 27 Eltahir HM, Blokpoel LA Ferreras, Jalal AR & Dixon JE, Direct contact-mediated non-viral gene therapy using thermo-sensitive hydrogel-coated dressings. *Biomater Adv*, 143 (2022) 213.