

Optimization of Nanoparticle Delivery of Nucleic Acids to Cells Using Poly(glycerol ester)

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secant group



MICHELIN



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**NEXT-GENERATION
DELIVERY INNOVATIONS**

Thank you for
joining us **and**
welcome



1. Overview of PGE as a transfection reagent
2. General performance
3. Improving results through use of Design of Experiments (DoE) and Machine Learning (ML)

Structural Tunability of PGE and Use of the Polyplex

End-group chemistry:

- tunes hydrophilicity
- incorporate targeting ligands

Diacid selection:

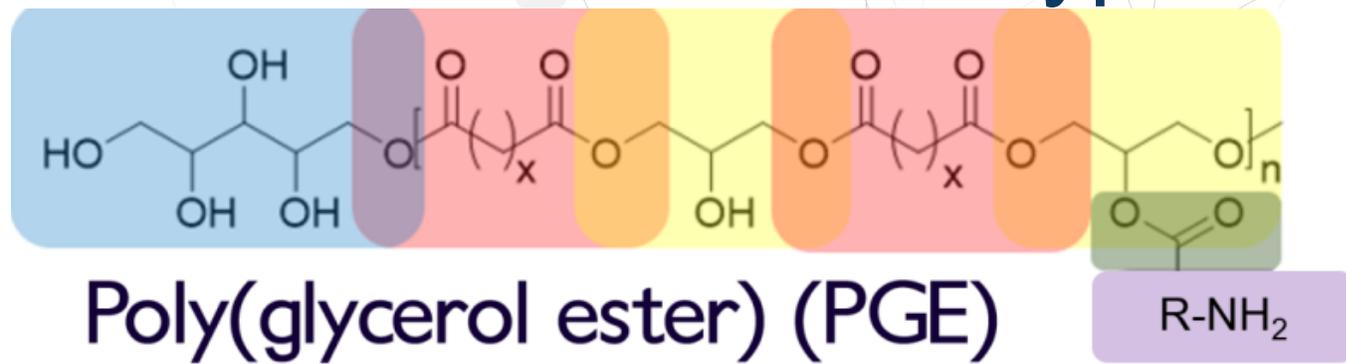
- tunes hydrophobicity and particle assembly
- impacts stability

Glycerol modification:

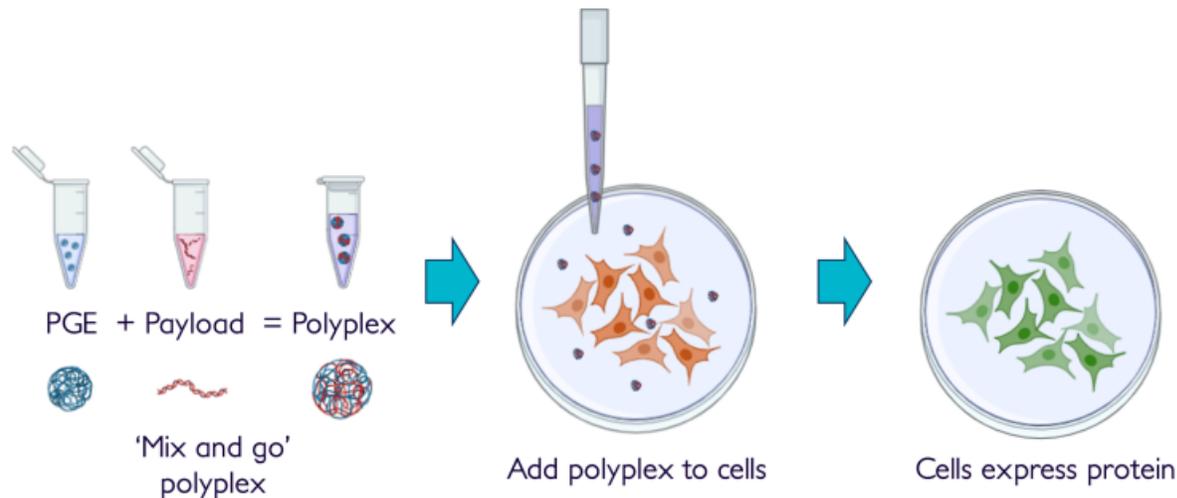
- controls branching
- provides site bioconjugation

Amine attachment:

- impacts complexation
- affects biocompatibility
- release of payload



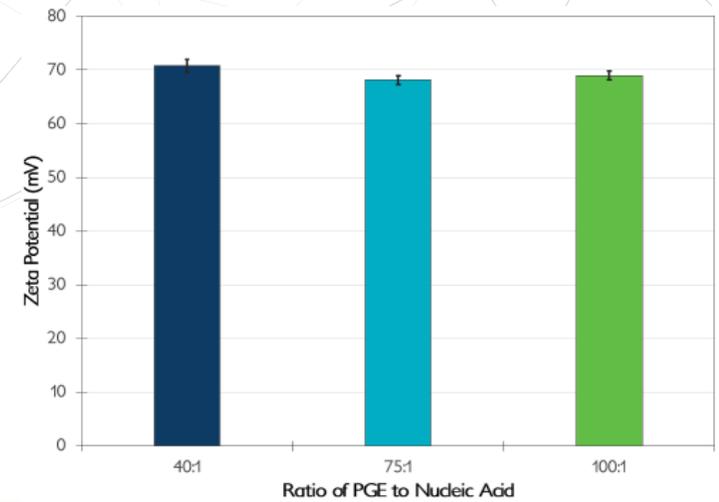
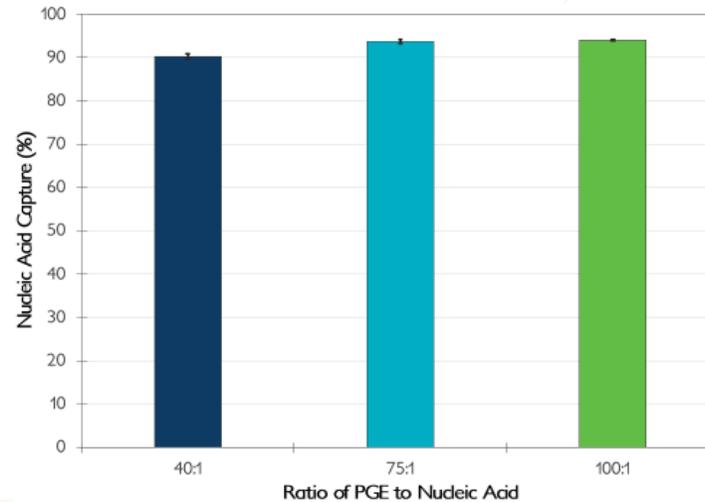
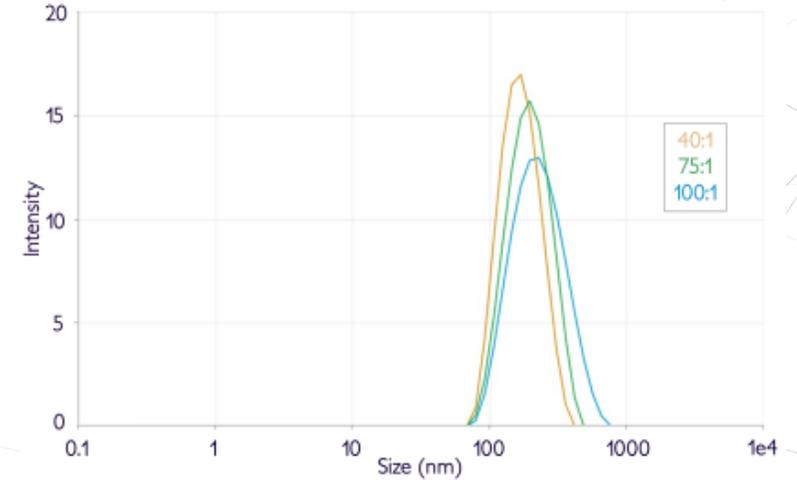
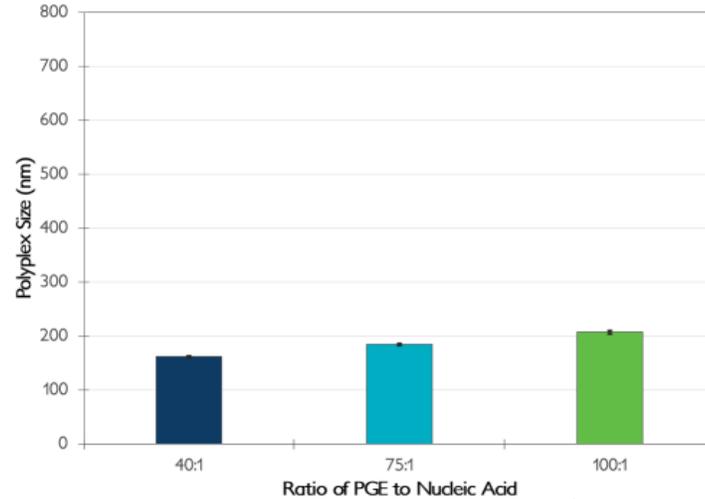
Poly(glycerol ester) (PGE)



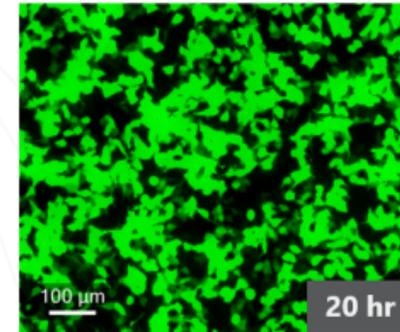
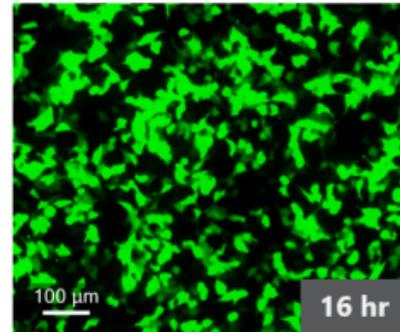
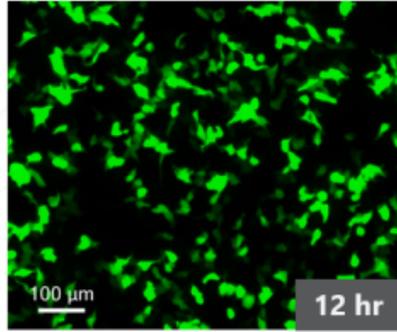
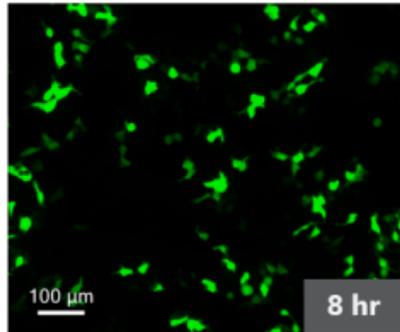
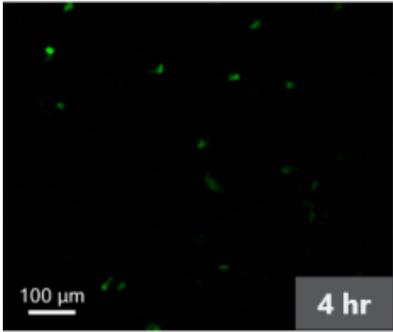
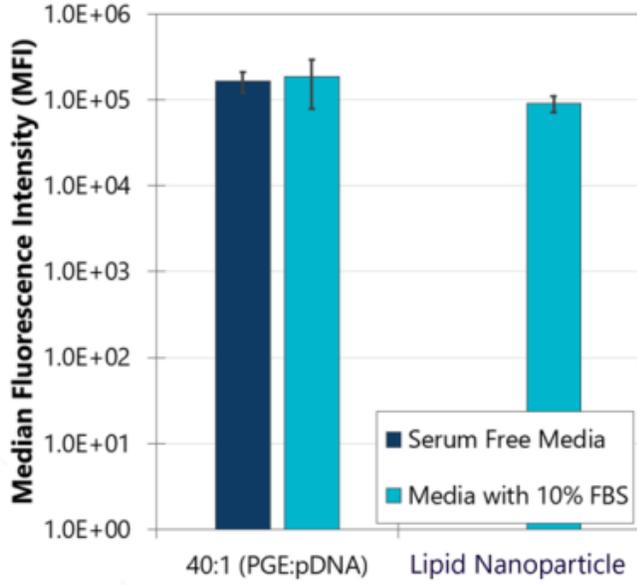
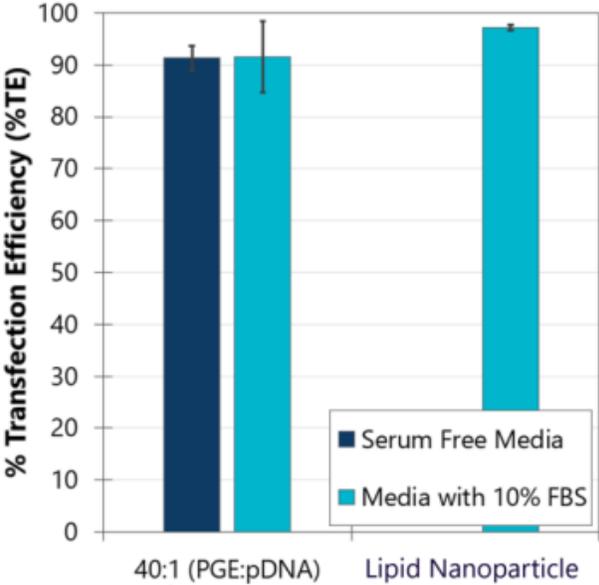
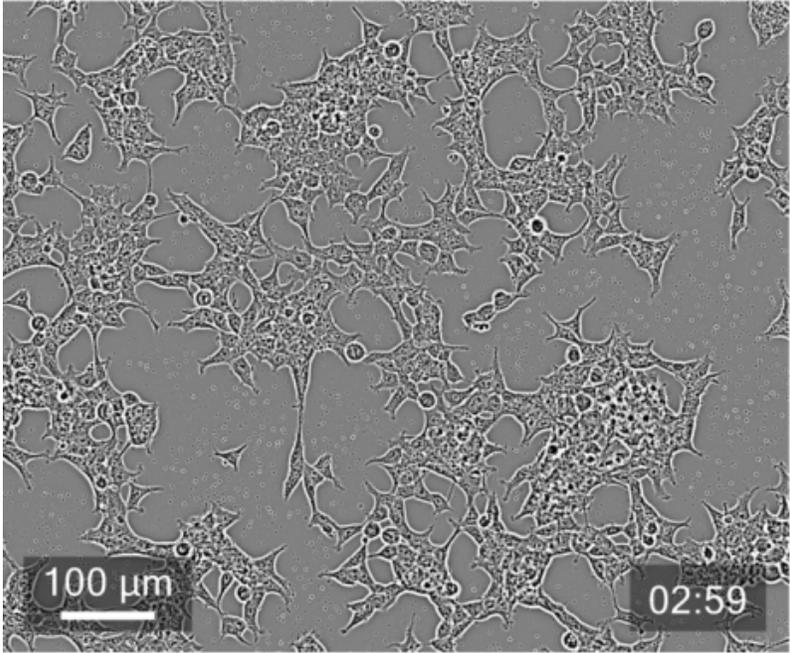
Poly(glycerol ester) (**PGE**) polyplex reagents are **single component**... no additives or enhancers required for transfection!

PGE Polyplex Properties Are Well Suited for Nucleic Acid Delivery

- Polyplex size when complexed with 1 μg pDNA is between 150 to 200 nm at PGE:pDNA mass ratios ranging from 40:1 to 100:1
- Payload capture (PicoGreen assay) remains above 90% for PGE at several ratios
- Zeta potentials are surprisingly high across the ratios tested



PGE Polyplexes Are Highly Effective at Delivering pDNA to HEK



DoE to Improve Delivery Effectiveness

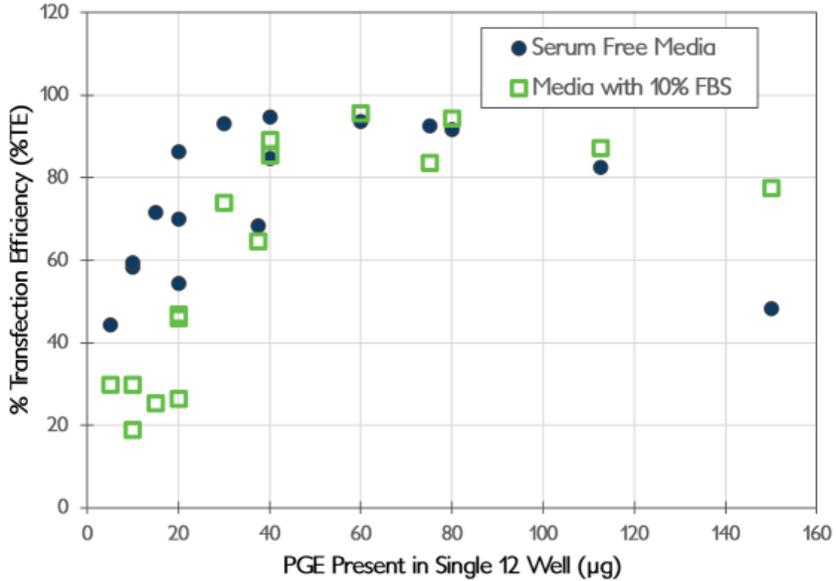
Factors:	Levels:
PGE:mRNA mass ratio	(4) - 10:1, 20:1, 40:1, 75:1
Media Conditions	(2) - Serum free, 10% FBS containing
mRNA Dosage per well (µg, 12 well plate)	(4) - 0.5, 1.0, 1.5, 2.0

- **Outputs (HEK293T cell testing)**
 - MFI
 - %TE
 - Cytotoxicity
- 64 total randomized runs

	RunOrder	PGE Ratio	Media Type	Dosage mRNA	MFI	%TE	%Dead
Group 1	1	40:1	Serum Free	0.5	88357.9	74.78	1.79
	2	75:1	10% FBS	0.5	80676.5	73.96	1.17
	3	75:1	Serum Free	1	457425	93.18	6.21
	4	75:1	Serum Free	1.5	538384	85.22	13.78
	5	20:1	Serum Free	2	291366	95.37	2.82
	6	10:1	Serum Free	1.5	85946.3	72.61	0.83
	7	40:1	10% FBS	0.5	16138.2	23.02	0.81
	8	75:1	Serum Free	2	114569	53.13	46.13
Group 2	9	20:1	10% FBS	1.5	124760	91.62	1.98
	10	20:1	Serum Free	0.5	50181	48.41	1.1
	11	10:1	10% FBS	2	47988.1	59.37	0.91
	12	20:1	10% FBS	1	24243.4	39.41	0.94
	13	20:1	Serum Free	1.5	165127	90.97	1.69
	14	40:1	Serum Free	2	496956	90.82	7.92
	15	10:1	Serum Free	0.5	28581.7	24.27	1.4
	16	75:1	Serum Free	0.5	33067.2	54.27	1.69
Group 3	17	40:1	Serum Free	1.5	409575	94.33	4.76
	18	10:1	10% FBS	0.5	8559.31	7.13	0.86
	19	10:1	10% FBS	1	12862.5	18.76	0.78
	20	40:1	Serum Free	1	94576.5	79.39	2.14
	21	40:1	10% FBS	1	111287	88.78	1.68
	22	40:1	10% FBS	1.5	1234749	95.56	3.48
	23	10:1	Serum Free	2	89603.6	79.4	1.61
	24	20:1	10% FBS	0.5	5790.25	2.72	0.95
Group 4	25	40:1	10% FBS	2	657184	94.57	5.31
	26	75:1	10% FBS	1.5	743947	92.33	7.54
	27	75:1	10% FBS	2	1025904	89.34	10.61
	28	75:1	10% FBS	1	61809.8	73.5	1.72
	29	20:1	10% FBS	2	91952	85.38	1.56
	30	10:1	Serum Free	1	21905.9	30.62	1.05
	31	20:1	Serum Free	1	44392.8	54.17	1.12
	32	10:1	10% FBS	1.5	11259.3	20.1	0.94
Group 5	33	10:1	Serum Free	2	154651	93.48	1.57
	34	20:1	10% FBS	1	47773.6	54.54	1.35
	35	40:1	10% FBS	0.5	27121.5	29.9	0.98
	36	10:1	10% FBS	2	21677.6	32.71	0.95
	37	10:1	Serum Free	1.5	81591.1	70.82	1.04
	38	75:1	10% FBS	1.5	494628	82.31	15.66
	39	10:1	10% FBS	1.5	23982.3	30.65	1.33
	40	40:1	Serum Free	2	373495	92.61	5.38
Group 6	41	40:1	10% FBS	1	132572	89.57	2.61
	42	10:1	10% FBS	0.5	44960.3	52.51	0.99
	43	20:1	Serum Free	1	34367.3	54.73	1.44
	44	75:1	Serum Free	1	263856	92.01	4.7
	45	75:1	Serum Free	0.5	143784	82.52	3.45
	46	75:1	Serum Free	2	20209.1	43.7	52.49
	47	20:1	10% FBS	0.5	32888.5	35.15	1.13
	48	20:1	Serum Free	0.5	5632.66	70.7	1.62
Group 7	49	10:1	Serum Free	0.5	10558.5	64.67	0.85
	50	20:1	Serum Free	2	649488	94.15	5.71
	51	40:1	10% FBS	1.5	323388	96.86	3.5
	52	75:1	10% FBS	0.5	35457.6	55.5	1.42
	53	20:1	10% FBS	2	88172.6	85.68	2.18
	54	75:1	10% FBS	1	347021	93.94	4.85
	55	40:1	Serum Free	1.5	218176	93.03	4.53
	56	10:1	10% FBS	1	22704.9	40.94	1.56
Group 8	57	75:1	10% FBS	2	343210	65.98	33.63
	58	10:1	Serum Free	1	6806.22	86.23	1.61
	59	40:1	10% FBS	2	527712	94.27	5.6
	60	20:1	10% FBS	1.5	32441.5	56.19	1.39
	61	20:1	Serum Free	1.5	194183	95.36	2.56
	62	40:1	Serum Free	0.5	52480.7	65.47	2.35
	63	75:1	Serum Free	1.5	494564	80.11	19.03
	64	40:1	Serum Free	1	83820.3	90.05	2.82

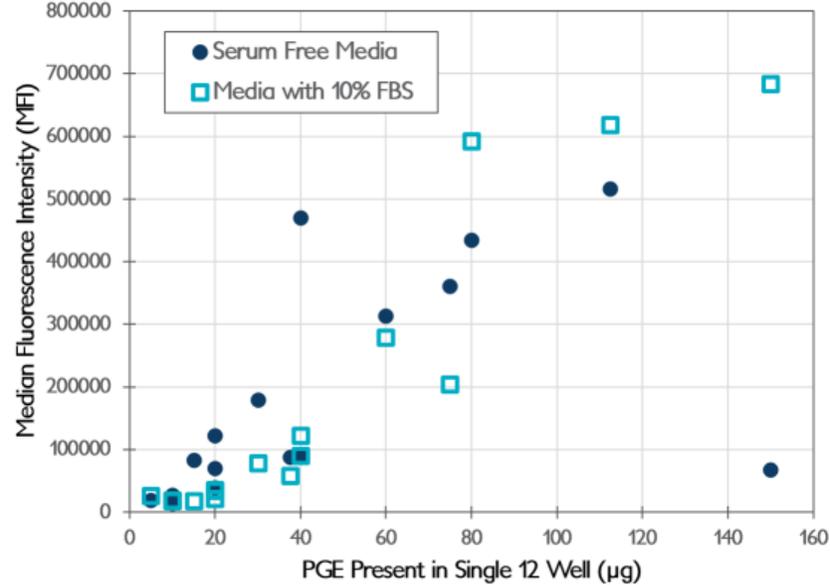
Impact of PGE Concentration on mRNA Expression

Impact of PGE Presence on mRNA Transfection



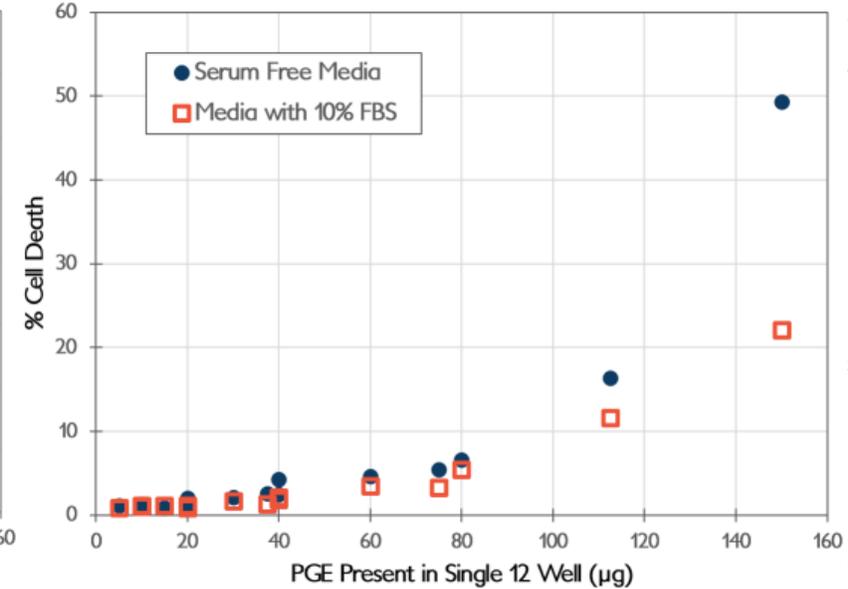
Transfection Efficiency (%TE) shows maxima at intermediate levels of PGE

Impact of PGE Presence on mRNA Transfection



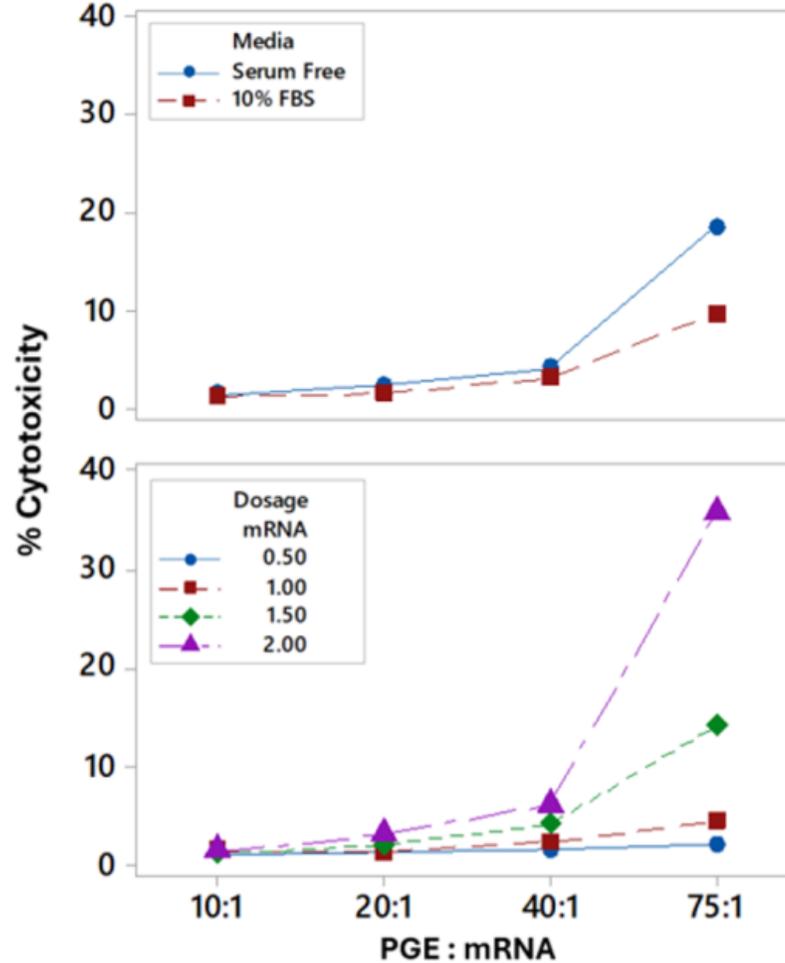
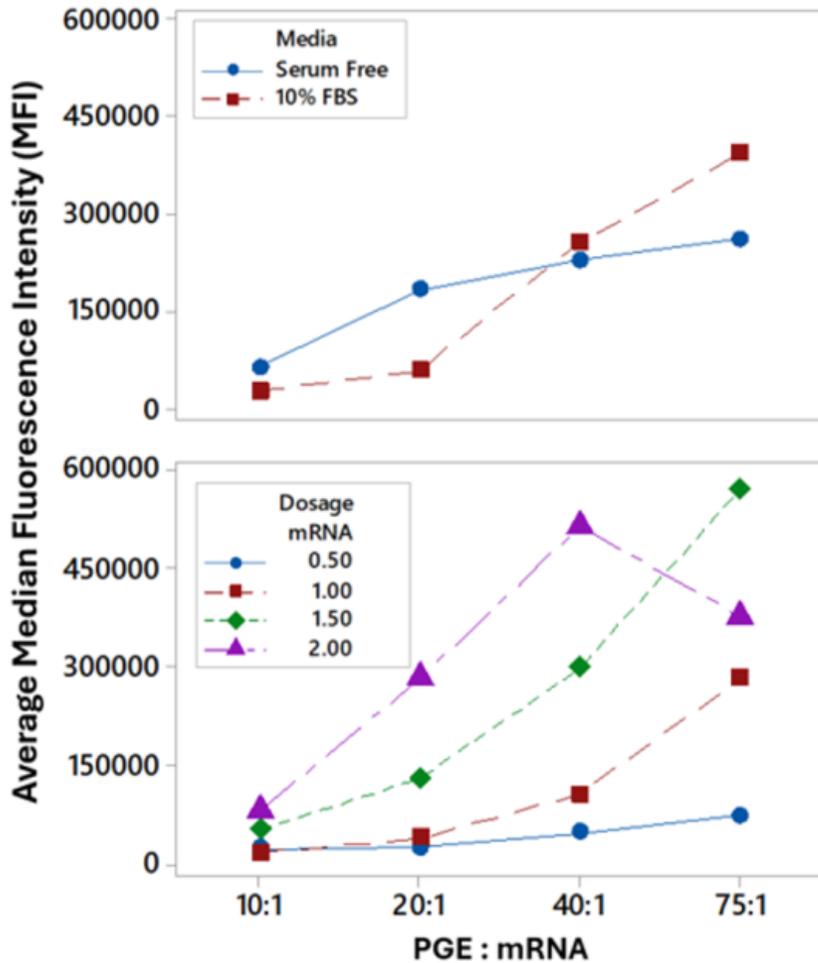
Median Fluorescence Intensity (MFI) increases as concentration of PGE increases until reaching levels of cytotoxicity.

Impact of PGE Presence on mRNA Transfection



Cytotoxicity behaves predictably as PGE concentration increases.

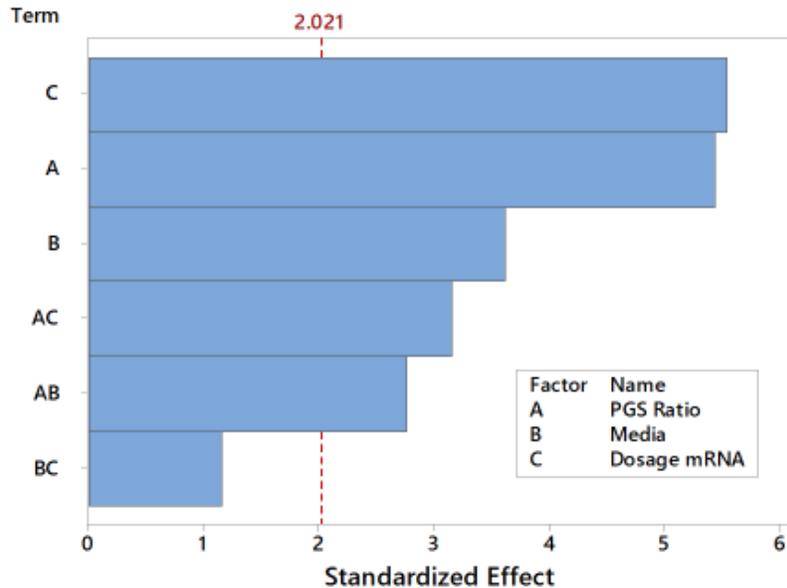
PGE Polyplexes Can Deliver mRNA in Serum-Containing Media



- Average %TE follows MFI
- MFI tends to improve as higher ratios are used in serum containing media
- Cytotoxicity is reduced at higher PGE : mRNA when serum is present
- MFI tends to increase with higher PGE : mRNA until high cytotoxicity occurs

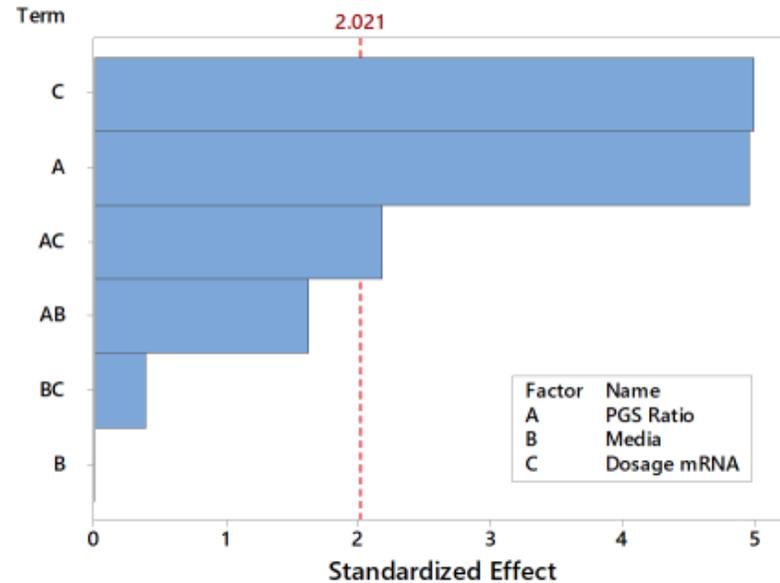
Standardized Factor Effects Show Parameter

%TE, $\alpha = 0.05$



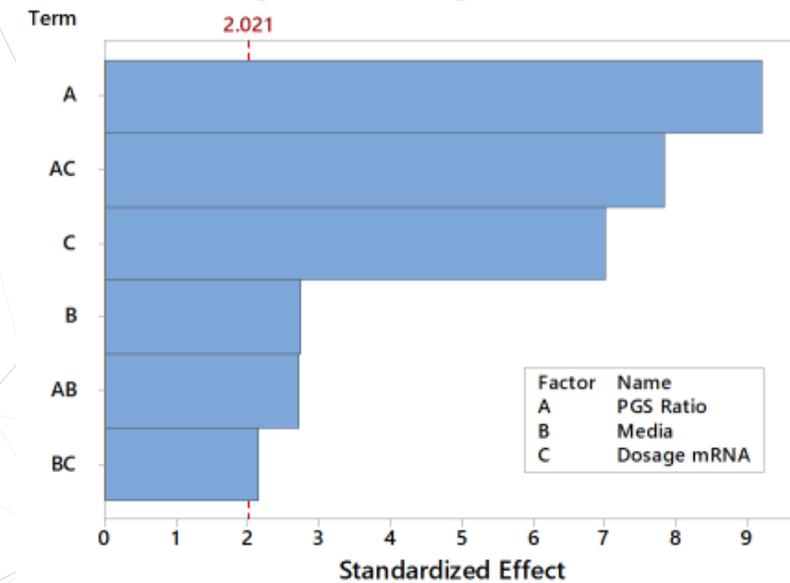
- All primary factors had significance
- Significant interactions were noted between Ratio*Dosage, and Ratio*Media type

MFI, $\alpha = 0.05$



- Dosage and ratio had significant effects
- Serum had no effect on MFI
- Significant interaction between ratio and dosage

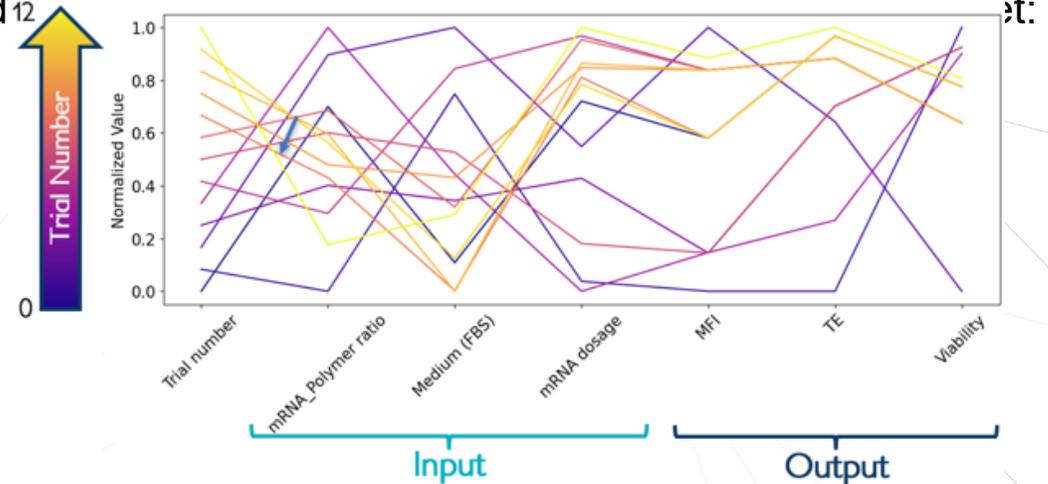
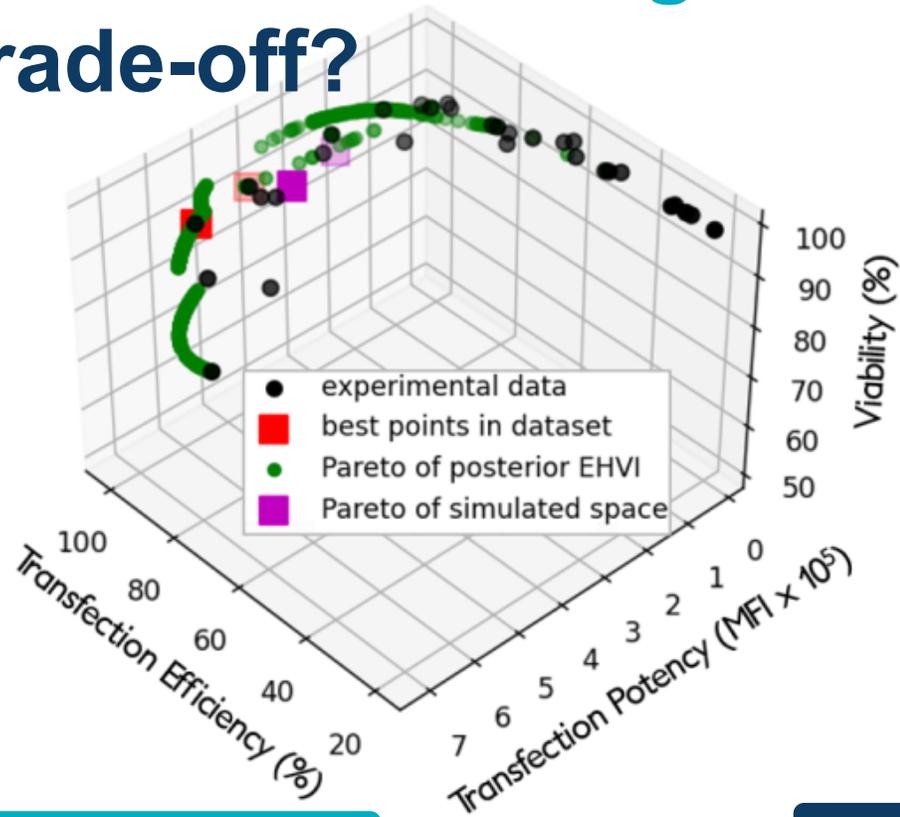
Cytotoxicity, $\alpha = 0.05$



- All primary and secondary factors had significance impact on cytotoxicity

Machine Learning and Bayesian Optimization: A better trade-off?

- Left:** Use of Multi Objective Bayesian Optimization using the DoE dataset to identify actual best points (red square) and build pareto front (green circle, purple square) with two methods to identify how close we are to maximizing MFI, %TE, and viability.
- Below:** Example of utilizing a Gradient Boosting Model in simulated space to identify optimal points:



DoE Results Best

PGS:mRNA Ratio	Media FBS %	Dosage mRNA (µg / well)	Viability (%)	% TE	MFI (10 ⁵)
40.0	10.0	2.0	94.5	94.4	5.9
20.0	0.0	2.0	95.7	94.8	4.7

Model Best

PGS:mRNA Ratio	Media FBS %	Dosage mRNA (µg / well)	Viability (%)	% TE	MFI (10 ⁵)
54.0	6.9	1.5	96.9	96.9	3.1
38.8	7.8	1.9	95.0	95.0	4.1

Conclusions:

Poly(glycerol esters) (PGEs) are a new polyplex reagent that can be used to deliver biologics.

- Cationic PGE can deliver both pDNA and mRNA.
- PGE can transfect cells in serum containing or serum free media.
- Design of Experiments (DoE) determined best polyplex conditions for transfection and most important factors impacting performance.
- Several machine learning models are being developed to better predict best polyplex conditions in fewer trials than using DoE.

We are actively looking for partners interested in this polyplex technology to address your specific needs and payloads!

Thank you for your time!

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The work presented today is part of a collaboration between Secant Group LLC. and Michelin

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