3D-Fect ™ transfection reagent – Results for gene silencing

3D-Fect™ is our newest transfection reagent specifically designed and developed for transfection of cells cultured in 3D scaffolds (sponges, matrices, inserts…). This formulation is based on a novel technology that allows adding a third dimension to cell cultures. 3D matrices not only add a third dimension to cells' environment, they also allow creating significant differences in cellular phenotype and behavior. Because 3D scaffolds are routinely used in basic research and therapeutic applications, OZ Biosciences has continued developing reagents for 3D applications. In this way, 3D matrices bearing complexes formed with ***3D-Fect™ reagent*** and siRNA are colonized by cells to be transfected in a more natural environment. ***3D-Fect™ reagent*** associated with 3D matrices allows high level of cell transfection in order to follow tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth…

Principal **3D-Fect™** advantages:

1. Highly efficient for gene silencing in 3D.
2. Ideal for any 3D scaffolds (sponges, matrices, insert)
3. Dedicated to short nucleic acid sequences (siRNA, miRNA…)
4. Completely biodegradable
5. Universal (primary cells and cell lines)
6. Simple, ready-to-use & rapid
7. Serum compatible
8. Long term gene silencing

Applications

3D-Fect™ reagent has been developed for very efficient transfection of siRNA and other small molecules into a wide variety of immortalized and primary cells cultured on 3D scaffolds. This transfection reagent is serum compatible and can be used for highly efficient gene silencing. This product is stable, ready-to-use and intended for research purpose only. The field of applications covers tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth, anticancer gene screening, cell survival, growth and differentiation, co-culture…

An updated list of transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

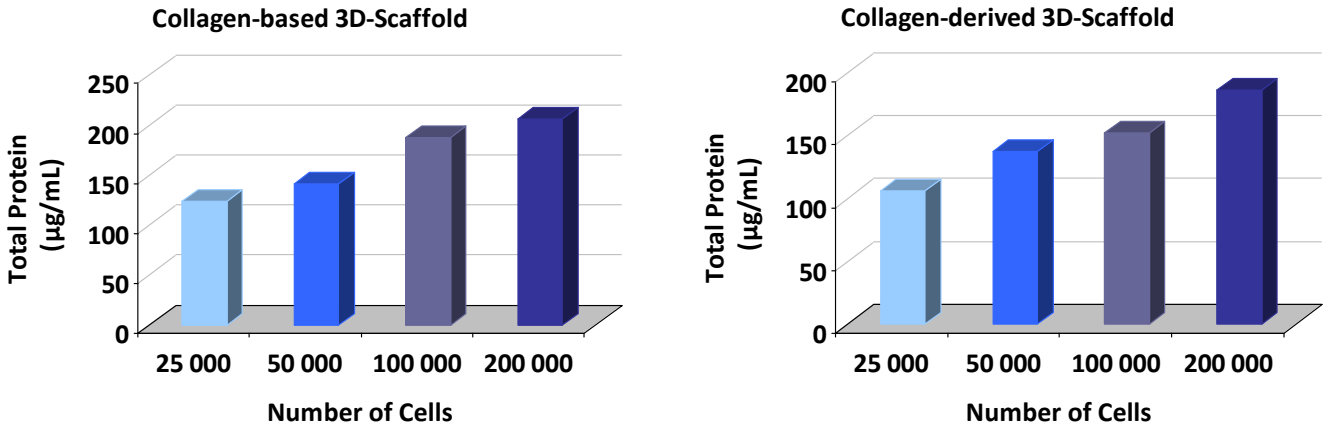
Scaffolds compatibility

Different 3D-Scaffolds can be used in association with ***3D-Fect™*** Transfection Reagent.

3D Scaffold	
Collagen	Collagen-based Scaffolds
Collagen-derived	Collagen-derived Scaffolds
HA	Hyaluronic Acid
Millicell™ (PTFE)	Cell culture insert (Millipore)
PCL	Polycaprolactone
PEG	Poly(Ethylene Glycol)
PLGA	Poly(lactic-co-glycolic acid)
PS	Poly(Styrene)
PU	Poly(Urethane)

3D-Scaffolds colonization

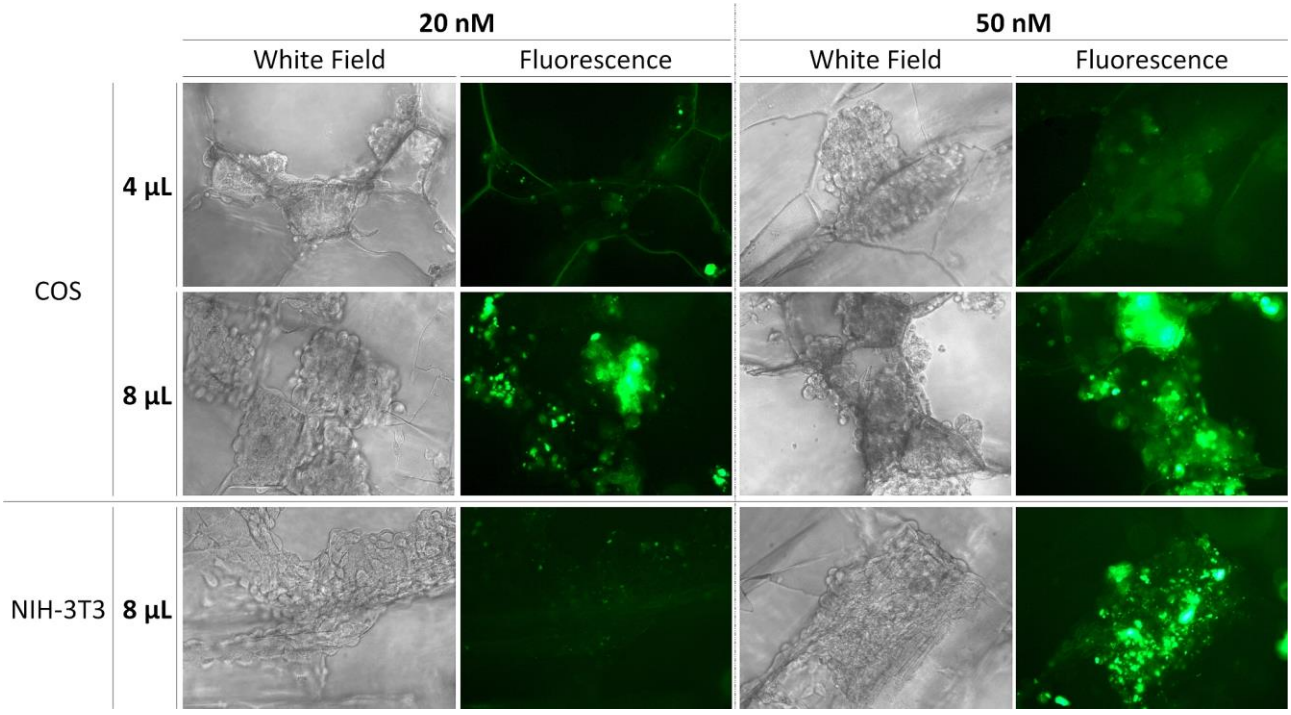
The third dimension added to cell culture allows higher cell number to be cultured. On a 24 well-plate, up to 200,000 cells can be seeded to colonize a 0.5 x 0.5 x 0.5 cm sponge.



25,000 to 200,000 cells were added on a 0.125 cm^3 Collagen-based or Collagen-derived 3D scaffolds. 48h after seeding, total protein amount was measured using Bradford kit (cat # BA00100). The protein quantity is proportional to the number of colonizing cells on 3D scaffolds. Depending on the scaffold volume, complexity and cell type, number of cells seeded may have to be adjusted.

3D-Fect™ transfection efficiency

In the following experiment, a fluorescently labeled siRNA was used as a positive control of delivery. 20 nM and 50 nM final concentration of siRNA were complexed to 4 and 8 µL of 3D-Fect™ transfection reagent. After 20 min incubation, atelocollagen scaffolds were hydrated with the complexes as described in the general protocol. 150,000 COS-7 and NIH-3T3 cells were finally added to the Gene Activated Matrix (GAM) and allowed to colonize the scaffold until evaluation of the transfection efficiency. Photos were taken under white field and fluorescence 48h after transfection.

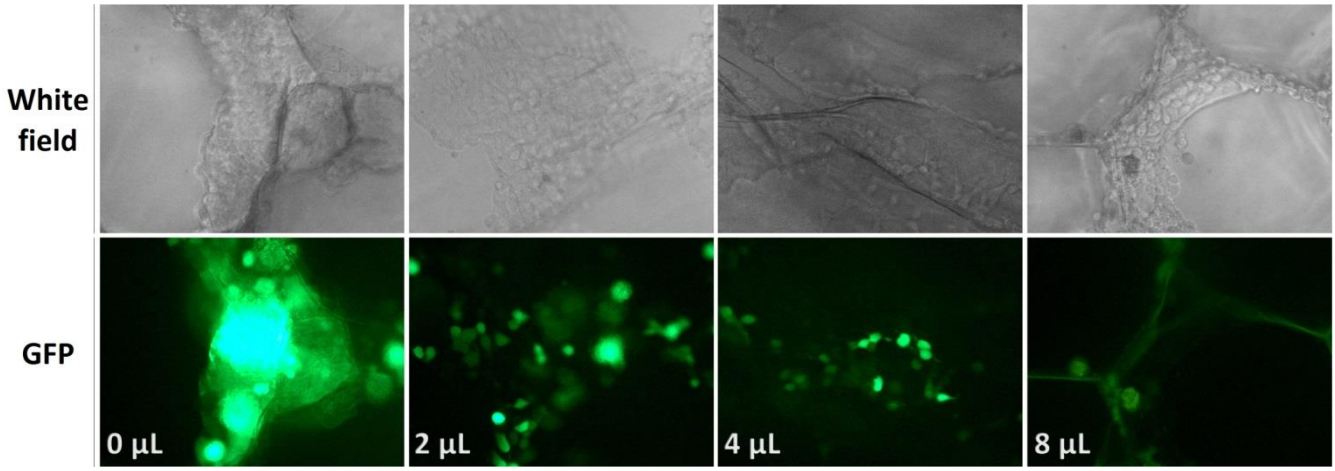


Results show that 3D-Fect™ allows to efficiently activate 3D-Scaffold for delivering siRNA into colonizing cells.

3D-Fect ™ Gene Silencing optimization

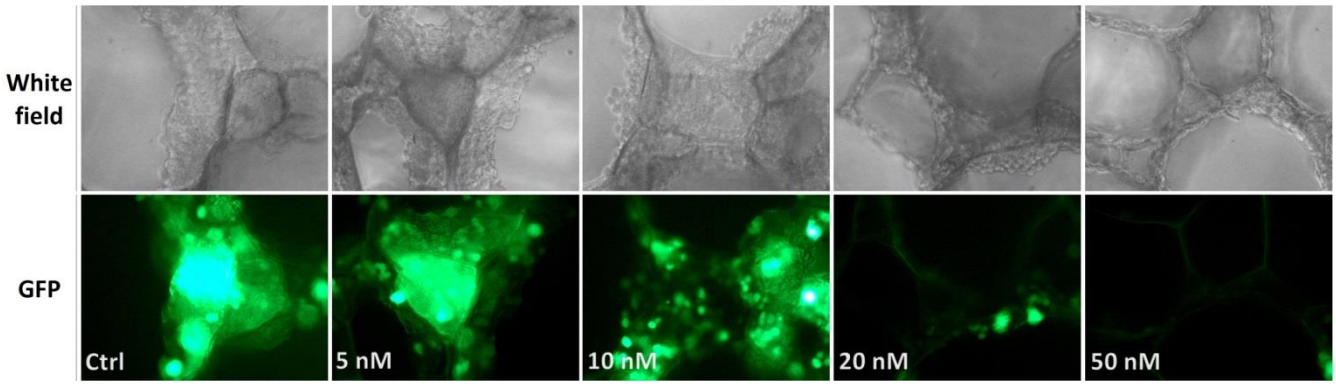
HeLa cell line stably transfected with GFP (HeLa-GFP) was used in this experiment.

A. Optimization of 3D-Fect™ volume. 50 nM final concentration of siRNA designed to silence GFP expression were complexed to several volumes of 3D-Fect transfection reagent. After 20 min incubation, atelocollagen scaffolds were hydrated with the complexes as described in the general protocol. 150,000 HeLa-GFP cells were finally added to the siRNA-GAM and allowed to colonize the scaffolds until evaluation of gene silencing efficiency. Photos were taken under white field and fluorescence 72h after transfection.



Results show that 3D-Fect™ allows efficient gene silencing in a volume dependent manner. Herein, 8µL of reagent are sufficient to obtain almost complete GFP extinction.

B. Optimization of siRNA quantity. Several concentrations of siRNA (0-50nMfinal) directed against GFP were complexed to 8 µL of 3D-Fect transfection reagent. After 20 min incubation, atelocollagen scaffolds were hydrated with the complexes as described in the general protocol. 150,000 HeLa-GFP cells were finally added to the siRNA-GAM and allowed to colonize the scaffolds until evaluation of gene silencing efficiency. Photos were taken under white field and fluorescence 72h after transfection.

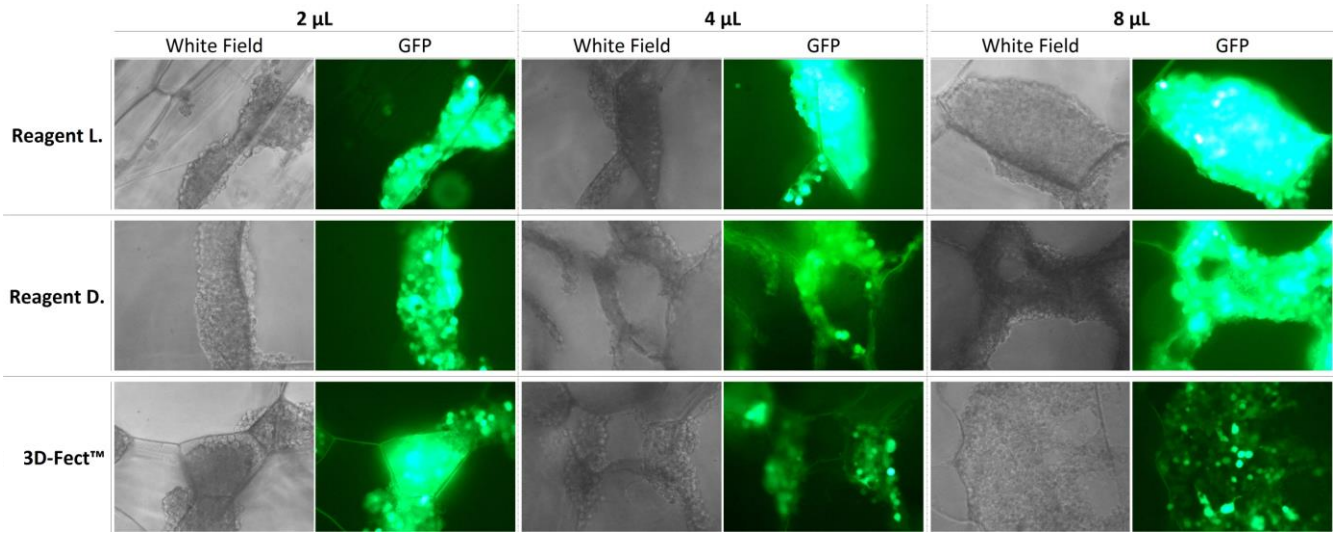


Results show that 3D-Fect™ allows efficient gene silencing in a siRNA dose dependent manner.

3D-Fect™ Gene Silencing, comparison with other reagents

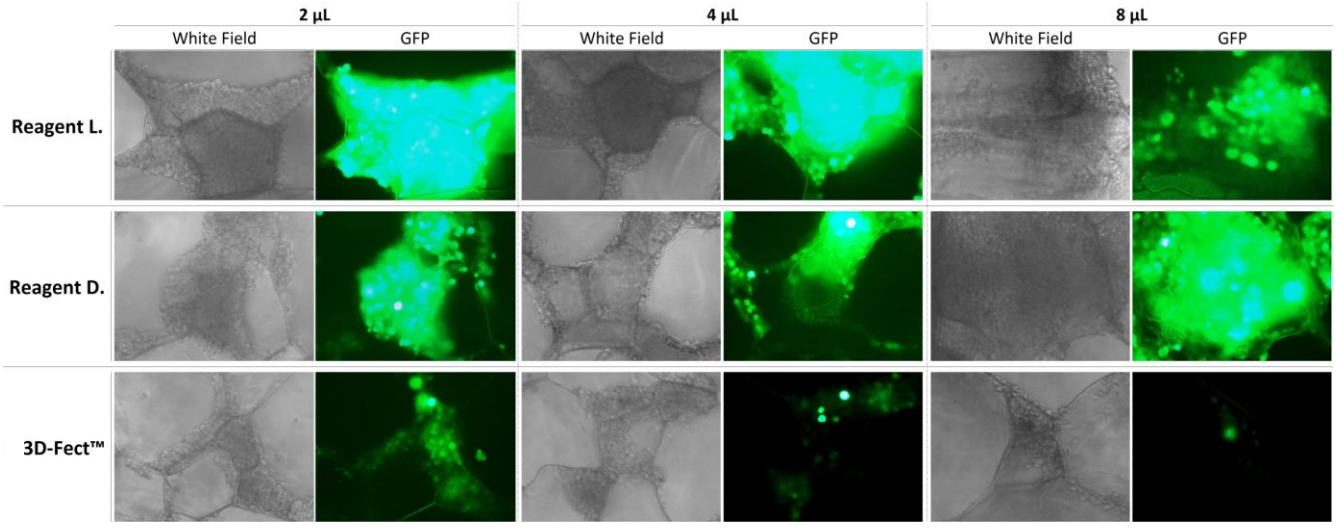
In these experiments, HeLa cell line stably transfected with GFP (HeLa-GFP) and two commercial transfection reagents dedicated to siRNA transfection in 2D (reagents L. and D.) were used.

A. **Comparison at very low concentration of siRNA.** 20 nM final concentration of siRNA designed to silence GFP expression were complexed to several volumes of 3D-Fect™ transfection reagent and compared to other commercial transfection reagents (L. and D.). After 20 min incubation, atelocollagen scaffolds were hydrated with the complexes as described in the general protocol. 150,000 HeLa-GFP cells were finally added to the siRNA-GAM and allowed to colonize the scaffolds until evaluation of gene silencing efficiency. Photos were taken under white field and fluorescence 72h after transfection.



Results show that only 3D-Fect™ allows efficient gene extinction at very concentration of siRNA

B. **Comparison at low concentration of siRNA.** The same experiment as described above was performed using 50 nM final concentration of siRNA.

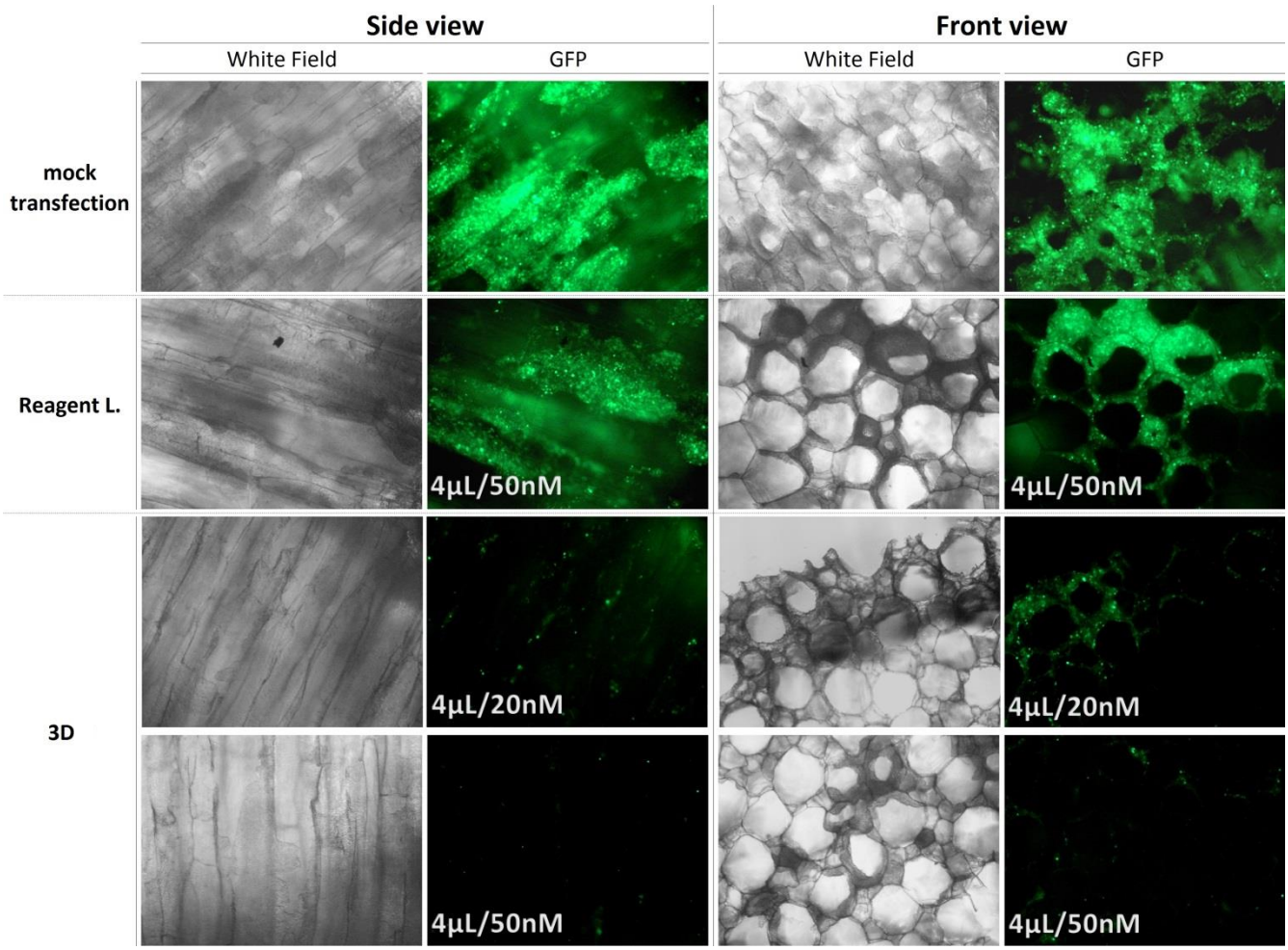


Results show that only 3D-Fect™ allows efficiently shutting down gene expression when low concentrations of siRNA are used.

C. Comparison using a low amount of reagent.

HEK-293 stably transfected with GFP plasmid were used to compare 3D-Fect efficiency to a commercial reagent routinely used in 2D cell culture for siRNA transfection with low amount of reagent.

20 nM and 50 nM final concentration of siRNA designed to silence GFP expression were complexed to 4µL of 3D-Fect™ transfection reagent and compared to other commercial transfection reagent (reagent L.). After 20 min incubation, atelocollagen scaffolds were hydrated with the complexes as described in the general protocol. 150,000 HEK-GFP cells were finally added to the siRNA-GAM and allowed to colonize the scaffolds until evaluation of gene silencing efficiency. Photos were taken under white field and fluorescence 72h after transfection.



Results show that:

- Only 3D-Fect™ allows shutting down gene expression with high efficiency
- Depending on the cell type, conditions may vary. In this case, 4µL of 3D-Fect are sufficient to silence GFP expression with a very high efficiency, whereas reagent L. proved inefficient under these conditions.

Bibliographic references

Please consult our list of references available on the website: www.ozbiosciences.com.