si3D-FectIN™ transfection reagent - Results

si3D-FectIN™ is the newest transfection reagent specifically designed and developed for **silencing gene expression** in cells cultured in Gel (or Hydrogel). 3D matrices not only add a third dimension to cells' environment, they also allow creating significant differences in cellular phenotype and behavior. Because 3D environments are routinely used in basic research and therapeutic applications, OZ Biosciences has continued developing reagents for 3D applications. Two new powerful reagents, ***si3D-FectIN™*** (for hydrogels) and ***si3D-Fect™*** (for scaffolds) are now dedicated to gene silencing. In this way, 3D matrices bearing complexes formed with ***si3D-FectIN™ reagent*** are colonized by cells to be transfected in a more natural environment. ***si3D-FectIN™ reagent*** associated with 3D matrices allows high level of cell transfection in order to follow 3D processes such as angiogenesis, tube and acini formation, colonization, neurite growth, tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation...

Principal **si3D-Fectin™** advantages:

1. Highly efficient for gene silencing in 3D
2. Ideal for any Hydrogel
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

si3D-FectIN™ reagent has been developed for very efficient transfection of siRNA and other small molecules into a wide variety of immortalized and primary cells cultured in 3D-hydrogels. 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 angiogenesis, tube and acini formation, colonization, neurite growth, tissue engineering, tissue regeneration, tumor invasion, neural differentiation, wound healing, cellular polarization, tissue formation...

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.

Hydrogels compatibility

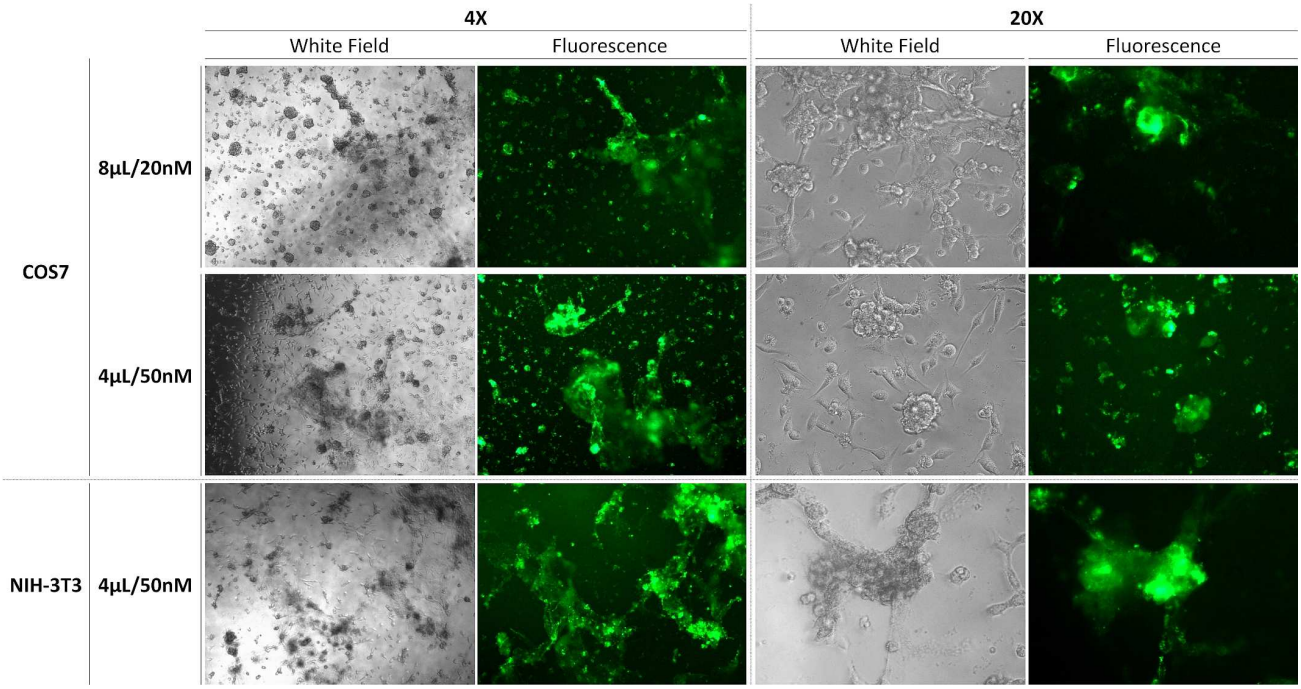
Different hydrogels can be used in association with **si3D-FectIN™** transfection reagent.

Examples of hydrogels compatible with ***si3D-FectIN™ transfection*** reagent.

Hydrogels	
Collagen	Collagen-Based Hydrogels
Collagen-Derived	Collagen-Derived Hydrogels
HA	Hyaluronic Acid
Gelatin	Extracellular Matrix (ECM)
Fibrin / Fibronectin	ECM
Fibrinogen	ECM
Laminin	ECM
Matrigel™	BD Bioscience
Poly-(Ethylene Glycol)	PEGylated hydrogels

si3D-FectIN™ transfection efficiency

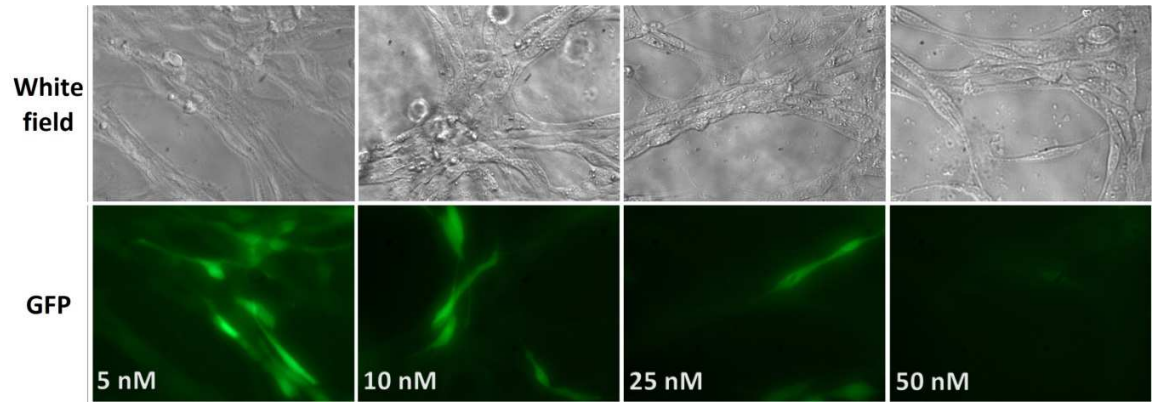
In the following experiment, a fluorescently labeled siRNA was used as a positive control of delivery. 20 and 50 nM final concentration of siRNA in 50 µL gel were complexed to 4 and 8 µL of si3D-FectIN™ transfection reagent. After 20 min incubation, complexes were loaded into an atelocollagen hydrogel before a 30 min polymerization period at 37°C as described in the general protocol. 30,000 COS-7 and NIH-3T3 cells were finally added onto the Gene Activated Matrix (GAM) and allowed to colonize the hydrogel until evaluation of the transfection efficiency. Photos were taken under white field and fluorescence 48h after transfection.



Results show that si3D-FectIN™ allows to efficiently activate Hydrogels for delivering siRNA into colonizing cells.

si3D-FectIN™ Gene Silencing optimization

NIH-3T3 cell line stably transfected with GFP (NIH-3T3-GFP) was used in this experiment.
Optimization of siRNA quantity. Several concentrations of siRNA (5-50nM final in 50 µL gel) directed against GFP were complexed to 4 µL of si3D-FectIN™ transfection reagent. After GAM formation as previously described, 30,000 NIH-3T3-GFP cells were added onto the hydrogel. Photos were taken under white field and fluorescence 48h after transfection.

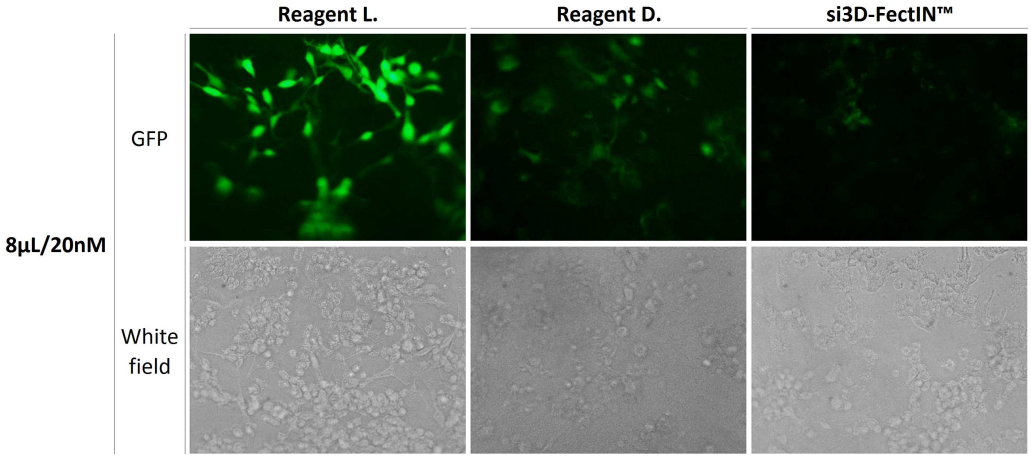


Results show that si3D-FectIN™ allows efficient gene silencing in a siRNA dose dependent manner.

si3D-FectIN ™ Gene Silencing, comparison with other reagents

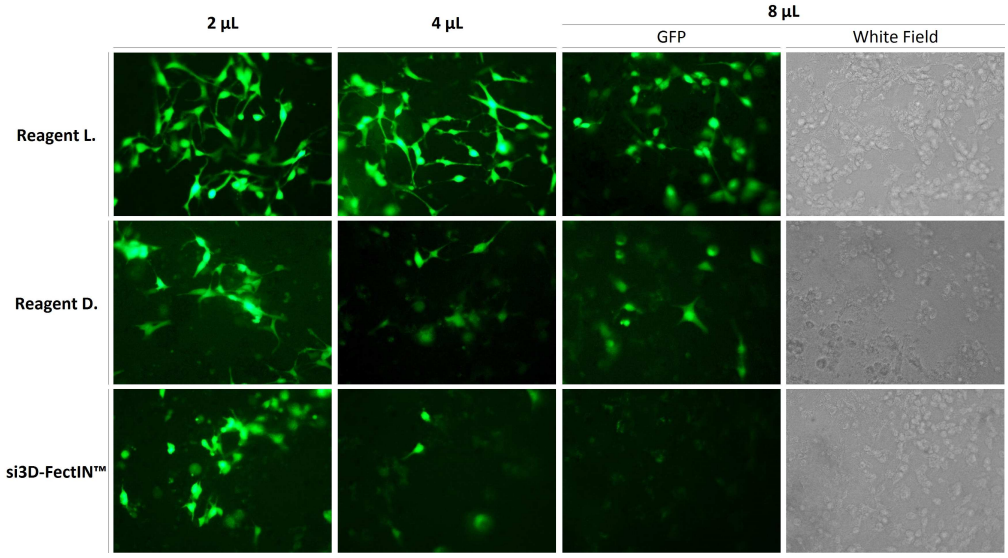
In these experiments, HeLa cell line stably transfected with GFP (HeLa-GFP) and two commercial transfection reagents dedicated to siRNA transfection in 2D (reagents L. and D.) were used.

A. Comparison at very low siRNA concentration (20 nM). 20 nM of siRNA designed to silence GFP expression was complexed 8 µL of si3D-FectIN transfection reagent and compared to other commercial transfection reagents (L. and D.). After 20 min incubation, complexes were loaded into 50 µL atelocollagen hydrogel before a 30 min polymerization period at 37°C as described in the general protocol. 30.000 HeLa-GFP cells were added onto the Gene Activated Matrix (GAM) and allowed to colonize the hydrogel until evaluation of the gene silencing efficiency. Photos were taken under white field and fluorescence 72h after transfection.



Results show that **si3D-FectIN**™ allows a more efficient gene silencing at very low concentrations of siRNA

B. Comparison at low siRNA concentration (50 nM). The same experiment as described above was performed using the recommended siRNA concentration and two volumes of reagents.



Results show that only si3D-FectIN™ allows to efficiently silence gene expression when low concentrations of siRNA are used.

Bibliographic references

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