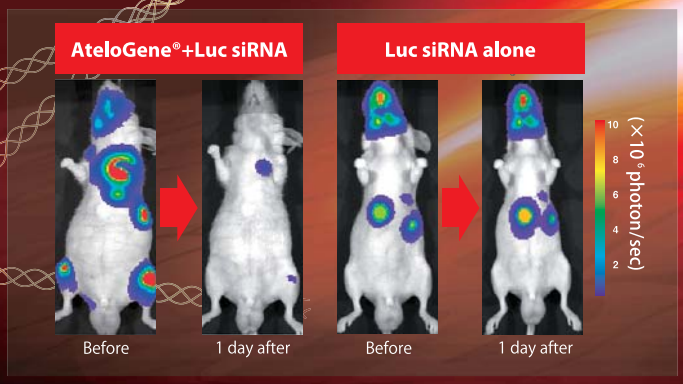
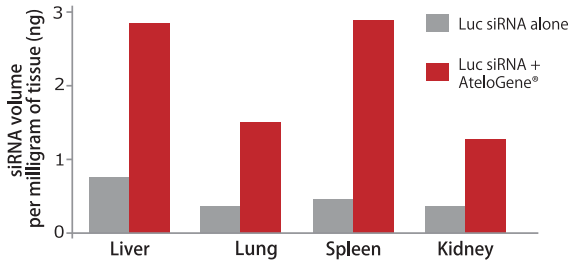
KOKEN

in vivo siRNA / miRNA Transfection Kits

AteloGene®

Local & Systemic Use





Amounts of siRNA detected in various tissues

AteloGene® efficiently delivers nucleic acids to different organs

With AteloGene® Systemic Use

Inhibition of luciferase expression by Luciferase siRNA administration

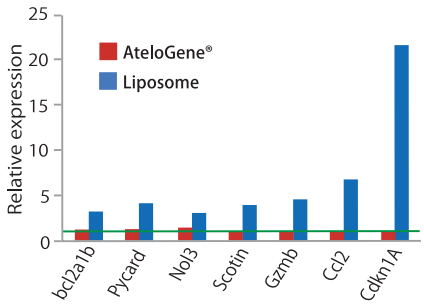
Luciferase siRNA(Luc siRNA)+AteloGene® was administered to a systemic metastatic model of prostate cancer cells stably expressing luciferase via the tail vein, and one day later the imaging device (IVIS) was used to confirm the delivery efficacy of siRNA. Measurements of tissue siRNA levels also confirmed that siRNA was delivered more efficiently to individual organs in the AteloGene®-treated group compared with siRNA alone.

(See Reference 1)

AteloGene® is clearly effective in introducing nucleic acids because of less virulence-induced changes in gene expression

With AteloGene® Systemic Use

Comparison of hepatotoxicity using microarrays



Expression levels of major apoptosis-related genes

Variations in the expression levels of various genes in the liver were analyzed by microarray 24 hours after administration of AteloGene® or Liposome into the tail vein of mice.

AteloGene® treatment group :
The number of genes whose expression level fluctuated or the fluctuation range were obviously little in comparison with the Liposome administration group, and it was shown that AteloGene® was suitable for the in vivo nucleic acid introduction.

Liposome treatment group :
Expression levels of apoptosis-related genes, such as Cdkn1A and Ccl2, and genes associated with biostimulatory / defensive / viral / stress / immune / wound responses were varied, suggesting high virulence.

(See Reference 2)

COSMO BIO CO., LTD.

AteloGene®
Local & Systemic Use

in vivo siRNA/miRNA transfection Kits AteloGene® Local & Systemic Use

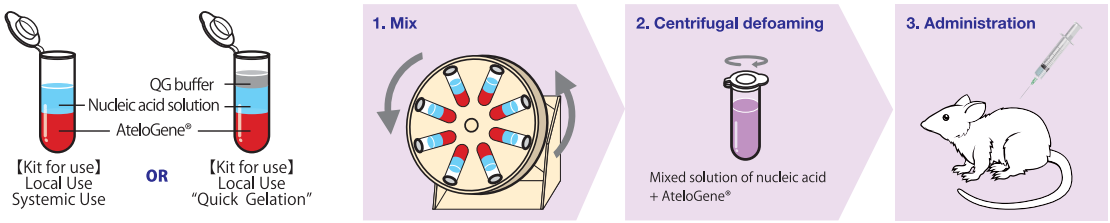
Characteristics of AteloGene®

- **Forms Complexes with nucleic acids to protect nucleic acids from degradation**
- **Suppresses immune response to double-stranded RNA**
- **Less variation in gene expression due to toxicity and clear nucleic acid transfer effects**
- **Possible to select either "Local Use" for sustained release of nucleic acids from gels or "Systemic Use" for whole body delivery without gels.**
- **AteloGene®Local Use "Quick Gelation" improves gelation rate, introduction efficiency, and convenience over conventional products.**

Atelocollagen, the major component of AteloGene®, forms complexes suitable for *in vivo* transfer by mixing nucleic acids in appropriate concentrations and proportions. Combination prevents the degradation of nucleic acids by nucleolytic enzymes and is efficiently introduced into tissue cells in *in vivo*. AteloGene®Local Use 2 products for topical administration retain nucleic acids at the site of administration because of their *in vivo* gelation properties, resulting in sustained release. On the other hand, the AteloGene®Systemic Use for systemic administration is designed to prevent the formation of gels and thus delivers nucleic acids efficiently to the bloodstream through the tail vein.

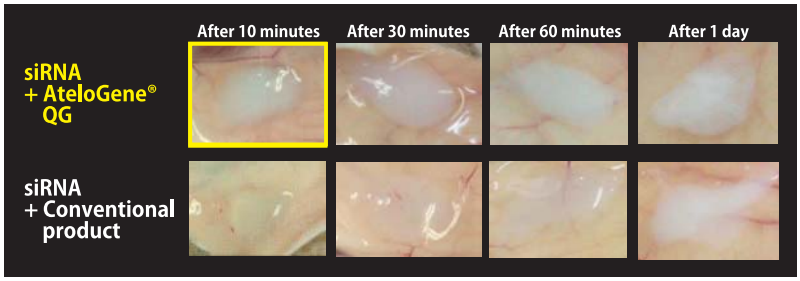
How to use

The preparation of AteloGene® is very simple. A mild mix of the nucleic acid solution with the AteloGene® solution can be administered immediately, with a concentration of 0.5 - 1.0 nmol for topical administration and 2.0 - 4.0 nmol for systemic administration.



AteloGene® Local Use gels *in vivo*, providing sustained-release nucleic acids

Comparison of gel formation time under mouse skin



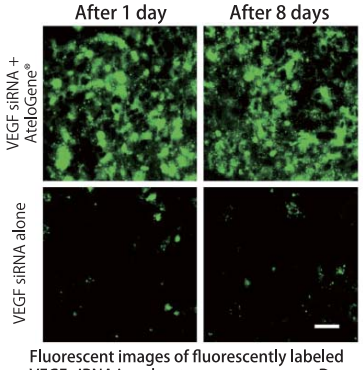
AteloGene®Local Use "Quick Gelation" (AteloGene® QG) and AteloGene®Local Use (conventional) were each mixed with siRNA and injected subcutaneously into mice to observe gelation. The results showed that AteloGene® QGs rapidly form gels after administration compared to the conventional one.

(Internal data)

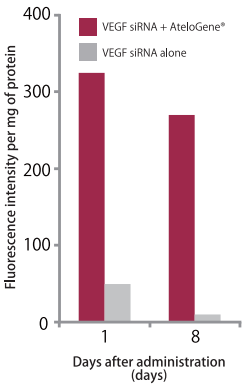
Nucleic Acids form Complexes with AteloGene® to protect nucleic acids from degradation

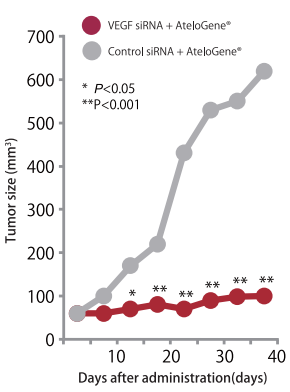
With AteloGene® Local Use

Suppressive effects of tumour growth by topical administration of VEGF siRNA



Fluorescent images of fluorescently labeled VEGF siRNA in subcutaneous tumors on Days 1 and 8 after administration





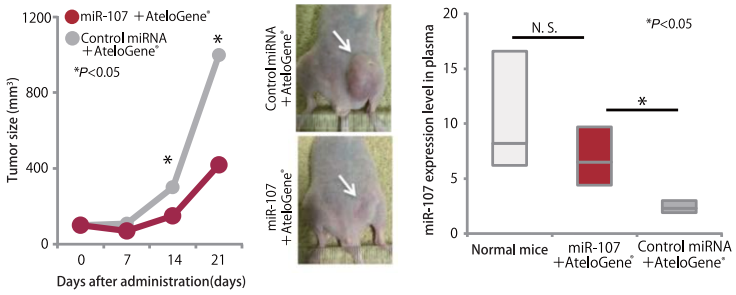
Local administration of fluorescently labeled VEGF siRNA+AteloGene® to subcutaneous tumors was shown to induce siRNA into tumors more effectively than when administered siRNA alone. Also, the fluorescent signal of siRNA introduced into the tumors was stably observed after 8 days (left, middle panel). In addition, significant suppressive effects of tumour growth were also observed with the same siRNA treatment (right panel).

(See Reference 3)

AteloGene® is also widely used in cancer research

Tumor growth suppression by local administration of miR-107

With AteloGene® Local Use "Quick Gelation"



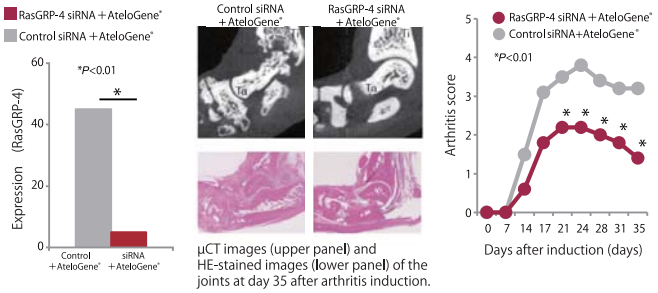
Administration of miR-107 plus AteloGene® (once weekly × 4 times in total) around subcutaneous tumors in pancreatic cancer models resulted in approximately 60% tumor growth inhibition on days 14 and 21 after treatment (left panel). In addition, miR-107, which is repressed in pancreatic cancer patients or mice, increased to levels similar to those in normal mice (right panel).

(See Reference 4)

AteloGene® is also suitable for nucleic acid transfer into joints

Improvement of arthritis with topical RasGRP-4 siRNA

With AteloGene® Local Use



μCT images (upper panel) and HE-stained images (lower panel) of the joints at day 35 after arthritis induction.

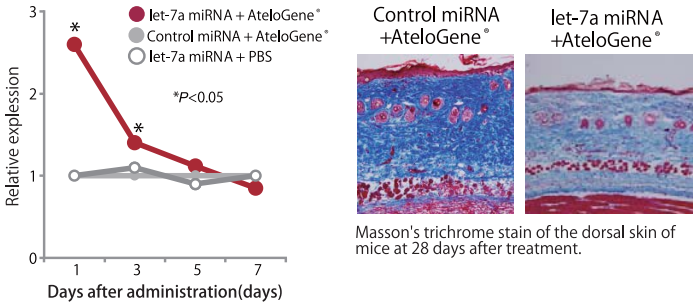
Administration of RasGRP-4 siRNA +AteloGene® (once at 14 days post-arthritis induction) into the ankle joint of rats in a collagen-induced arthritis model inhibited RasGRP-4 protein expression by approximately 80% at 35 days post-induction (left panel). In addition, the progression of joint destruction was inhibited (middle panel) and the arthritis score was greatly improved (right panel).

(See Reference 5)

AteloGene® also helps to introduce nucleic acids into the skin

Inhibition of bleomycin-induced dermal fibrosis by intraperitoneal administration of Let-7a miRNA

With AteloGene® Systemic Use



Masson's trichrome stain of the dorsal skin of mice at 28 days after treatment.

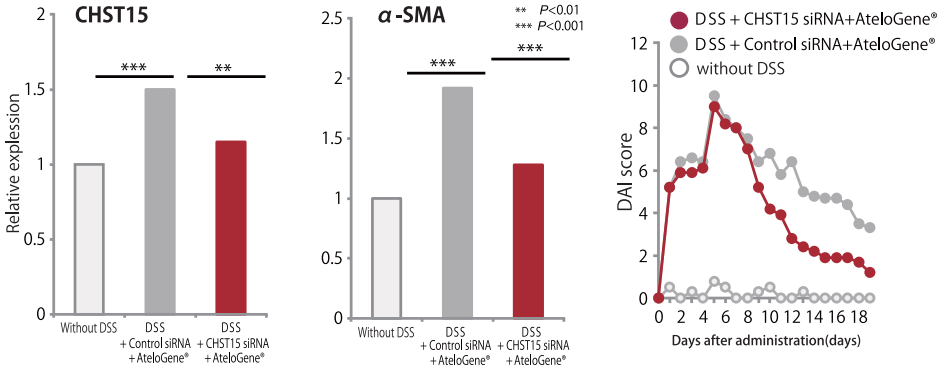
Intraperitoneal administration of let-7a miRNA+AteloGene® to mice significantly increased the expression of let-7a miRNA in dorsal skin up to 3 days later. Furthermore, intraperitoneal administration of let-7a miRNA+AteloGene® (once weekly × 4 times in total) to mice in a bleomycin-induced scleroderma model inhibited skin thickening and collagen fiber proliferation at 28 days after administration (right panel), demonstrating high nucleic acid transfer efficacy.

(See Reference 6)

AteloGene® also helps to introduce nucleic acids into the digestive system

Amelioration of colitis by intraperitoneal administration of CHST15 siRNA

With AteloGene® Systemic Use



When CHST15 siRNA+AteloGene® was intraperitoneally administered to a dextran sodium sulfate (DSS)-induced colitis model (every 4 days from day 6 after induction × 4 times in total), CHST15 and alpha-SMA expression was suppressed to the same extent as in the non-DSS group on day 19 after induction (left panel, center panel) and disease (DAI) scores were halved (right panel).

(See Reference 7)

AteloGene®
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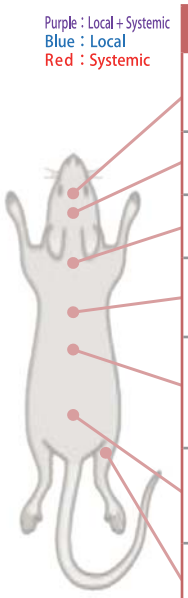
List of atelocollagen DDS-related reports (Multiple reports are merged into each disease model)



organ	si/shRNA	miRNA/miRNA inhibitor	antisense ODN*1/SSO*2 Decoy aptamer	plasmid DNA/Vector (siRNA/miRNA/Virus)
Head and neck, Eye, Oral cavity	Oral squamous cell carcinoma; Choroidal neovascularization; Head and neck cancer; Salivary adenoma; Dry eye	Oral squamous cell carcinoma; laryngeal cancer; Head and neck cancer; Undifferentiated large cell carcinoma	-	Corneal injury
Brain, Nerves, Spinal cord	Progressive multifocal leukoencephalopathy; Gioma; Nuroblastoma; Spinal cord injury; Hippocampal function; Multiple sclerosis	Sciatica, Glioma	Familial amyloid polyneuropathy	Glioma
Respiratory organs	Non-small cell lung cancer; Asthma; Mesothelioma; Lung adenocarcinoma; Lung cancer; Emphysema	-	-	-
Cardiovascular	Abdominal aortic aneurysm; Intimal hyperplasia; Intraplaque hemorrhage; Lower extremity ischemia; Hypertension; Vasoconstriction; Ischemia-reperfusion injury	Vascular inflammatory disease; Cardiac hypertrophy; Atherosclerosis	-	Thoracic aortic aneurysm, Vascular endothelial dysfunction
Digestive organs	Hepatocellular carcinoma; Colon cancer; Pancreatic cancer; Bile duct cancer; Colorectal cancer; Esophageal squamous cell carcinoma; Drug-and chemical-induced liver injury; Scirrhous gastric cancer; Alcoholic hepatitis; Acute/chronic colitis	Pancreatic cancer; Colon cancer; Scirrhous gastric cancer; Esophageal squamous cell carcinoma; Fatty liver; Colorectal cancer	Digestive cancer; Rectal cancer	Scirrhous gastric cancer
Genital and urinary organs	Prostate cancer, Cervical cancer, Endometrial cancer, Testicular cancer, Urothelial cancer, Bladder cancer, Ovarian hyperstimulation syndrome, Ovarian cancer, Renal stones, Renal cell carcinoma	Prostate cancer; Bladder cancer; Endometrial Cancer	Prostate cancer, Testicular cancer	-
Locomotorium	Amyotrophy; Arthritis; Fracture; Bone differentiation; Osteosarcoma; Ewing's sarcoma; Muscle injury (Muscle regeneration) ; Limb-girdle muscular dystrophy	Arthritis; Refractory fracture; Anterior cruciate ligament injury; Meniscal injury; Medial collateral ligament injury; Achilles tendon injury; Muscle injury (Muscle regeneration)	Rhabdomyosarcoma, Osteosarcoma	Osteoporosis
Skin	Melanoma; Nickel allergy; Fibrosis; Epidermoid carcinoma; Scleroderma; Contact hypersensitivity	Scleroderma	Contact hypersensitivity	-
Immunity, Metabolism, etc.	Obesity; Breast cancer; Myeloma; Autoimmune diabetes; Aging	-	Sepsis	-

＊1 ODN: Oligodeoxynucleotide ＊2 SSO: Single strand oligonucleotide

Comparison table of AteloGene® products

Description	Formation of complexes with nucleic acids	Method of administration	Gel formation time	Concentration of nucleic acid	Dosing interval	Cat. No.	Quantity*3
AteloGene® Local Use "Quick Gelation"	Yes	Local	10 min	0.5 - 1.0 nmol/administration	Around 1week	1492	1 kit
AteloGene® Local Use	Yes	Local	30 min	0.5 - 1.0 nmol/administration	Around 1week	1392	1 kit
AteloGene® Systemic Use	Yes	Intravenous Intraperitoneal	Without gelation	2.0 - 4.0 nmol/administration	Around 3days	1393	1 kit

＊3 Cat. No. 1490: 15 times administration, Cat. No. 1390/1391: 10 times administration
It is calculated as 200 µl/dose, so it may be possible to administer more than the number of doses indicated depending on the target tissue.

Reference

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3. Takei Y, *et al.* A small interfering RNA targeting vascular endothelial growth factor as cancer therapeutics. (2004) *Cancer Res.* **64**(10): 3365-3370
4. Imamura T, *et al.* Depleted tumor suppressor miR-107 in plasma relates to tumor progression and is a novel therapeutic target in pancreatic cancer. (2017) *Scientific reports.* **7**(1): 5708.
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6. Makino K, *et al.* The downregulation of microRNA let-7a contributes to the excessive expression of type I collagen in systemic and localized scleroderma. (2013) *J Immunol.* **191**(8): 3905-3915.
7. Suzuki K, *et al.* Pivotal role of carbohydrate sulfotransferase 15 in fibrosis and mucosal healing in mouse colitis. (2016) *PLoS One.* **11**(7): e0158967.

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COSMO BIO CO., LTD.
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
TEL : (81)3-5632-9617
FAX : (81)3-5632-9618
e-mail : export@cosmobio.co.jp
URL : www.cosmobio.com