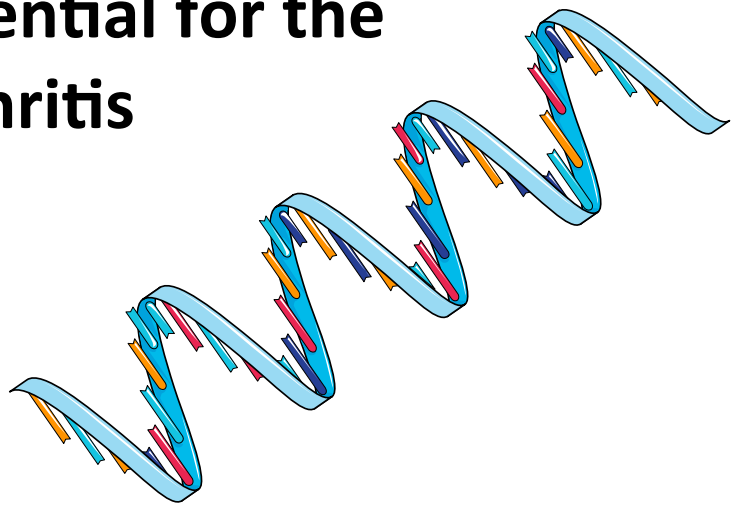
Synthetic mRNA-based approaches for tissue regeneration– Application potential for the treatment of osteoarthritis

Prof. Dr. rer. nat. Meltem Avci-Adali
University Hospital Tübingen
Dept. of Thoracic and Cardiovascular Surgery



Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs)

Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
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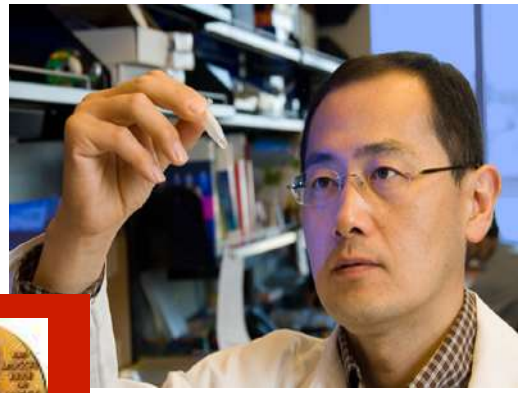
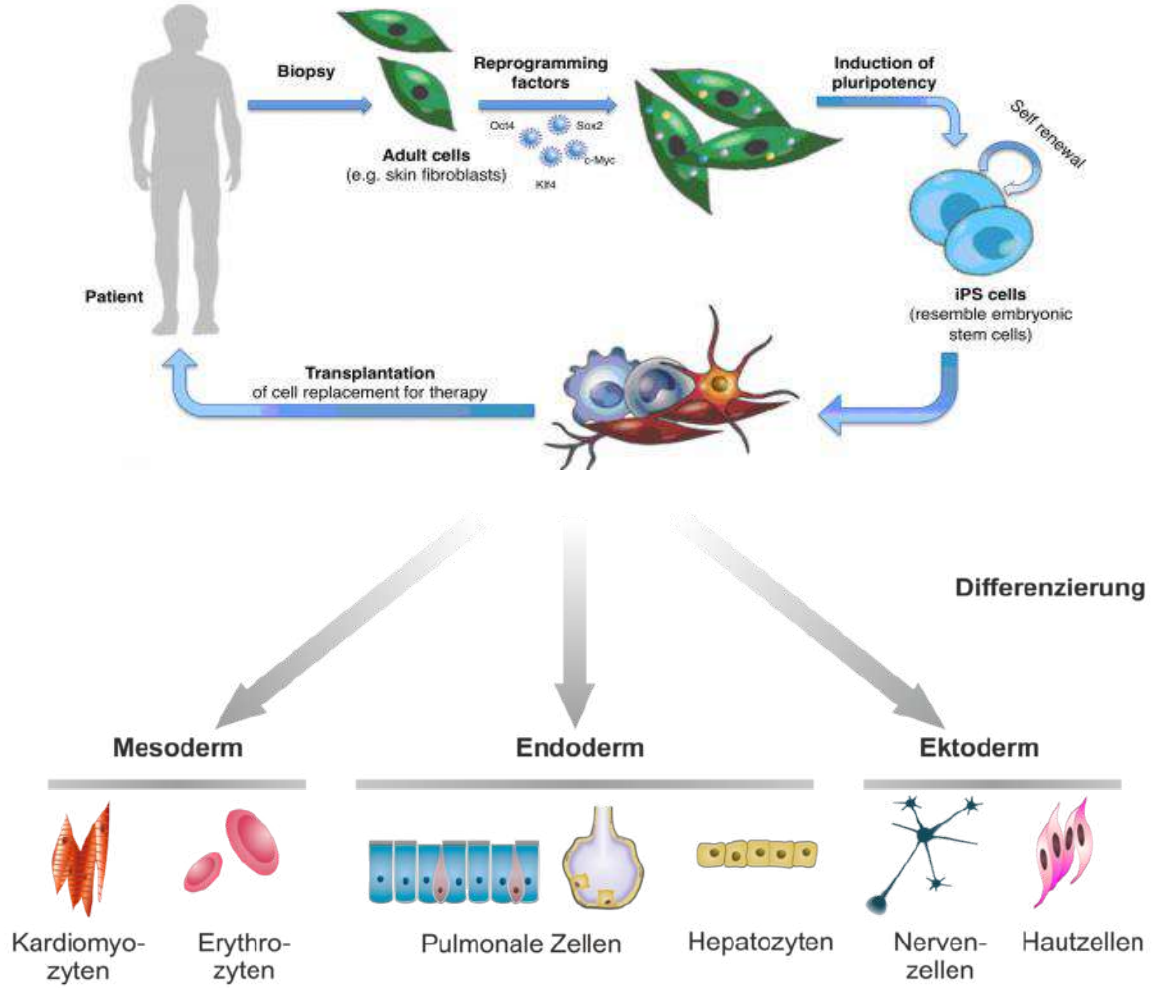
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024



Jointly with Sir John B. Gurdon
Nobel Prize 2012
in Physiology or Medicine

Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs)



Modifiziert aus http://www.lebao.de / http://www.eurostemcell.org/de

Disadvantages of these methods

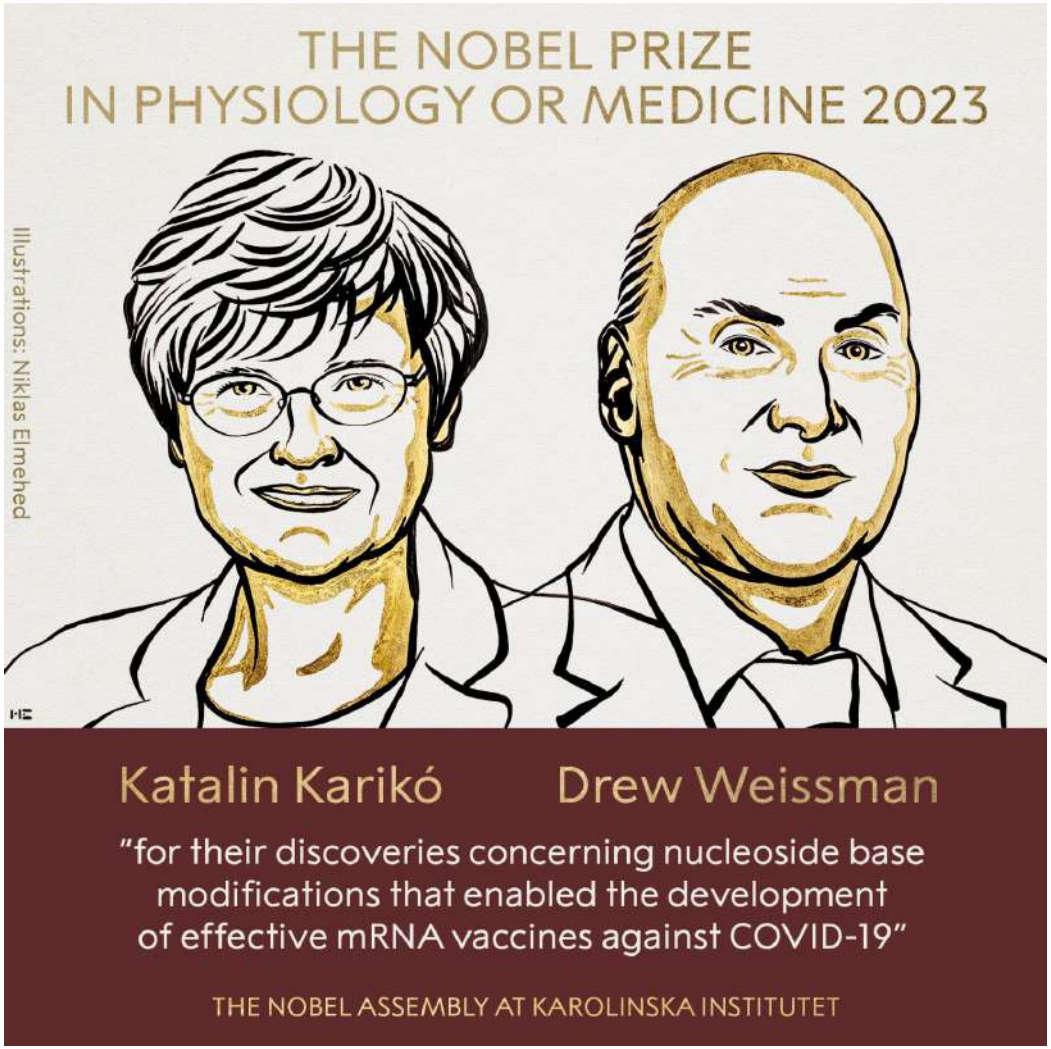
→ **Use of retroviral vectors**
→ Genome integration→ Induction of mutagenesis

Problem:
→ cannot be clinically applied

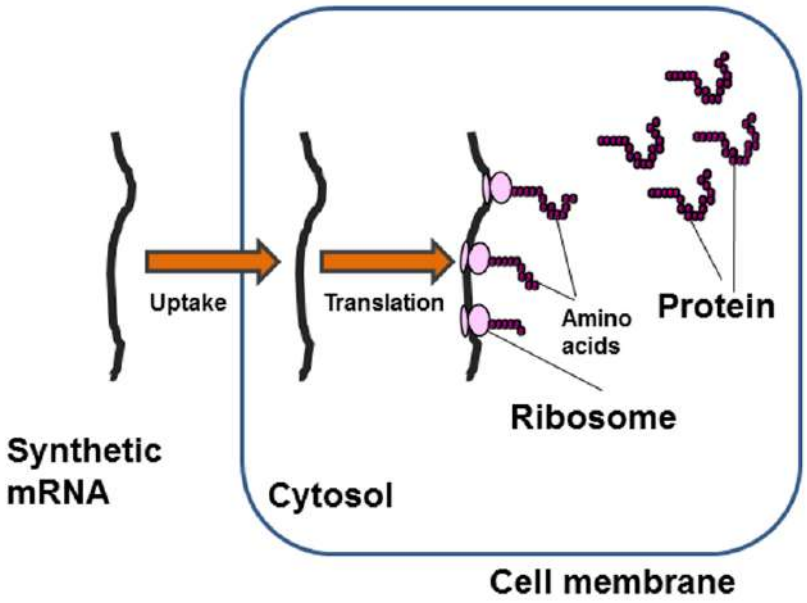
How can mutations be prevented?

Use of non-mutagenic molecules is required:
→ Synthetic mRNAs

Modified synthetic messenger RNA (mRNA)

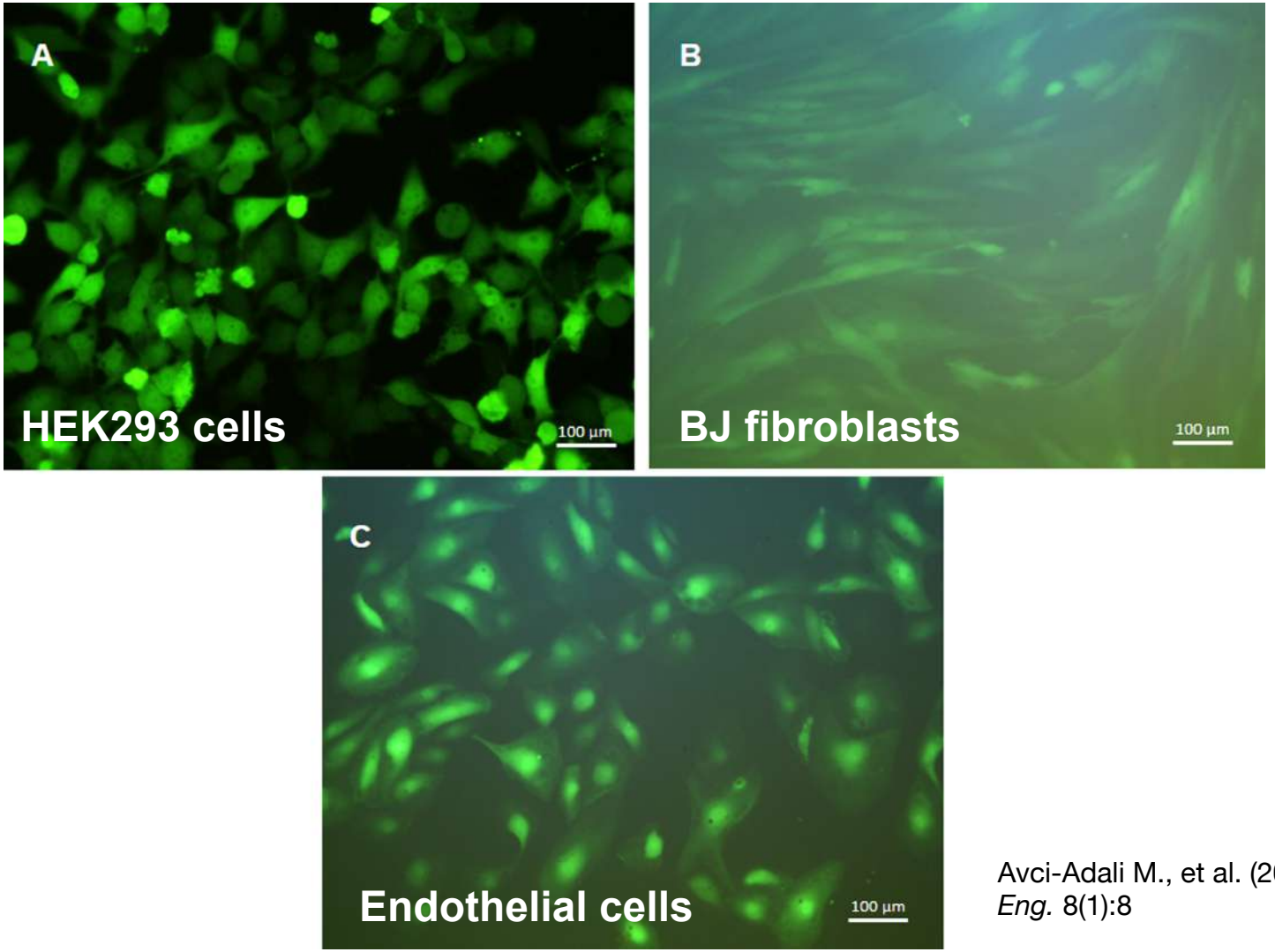


Exogenous delivery of modified synthetic messenger RNA (mRNA)



Avci-Adali M., et al. (2014) *J Biol Eng.* 8(1):8

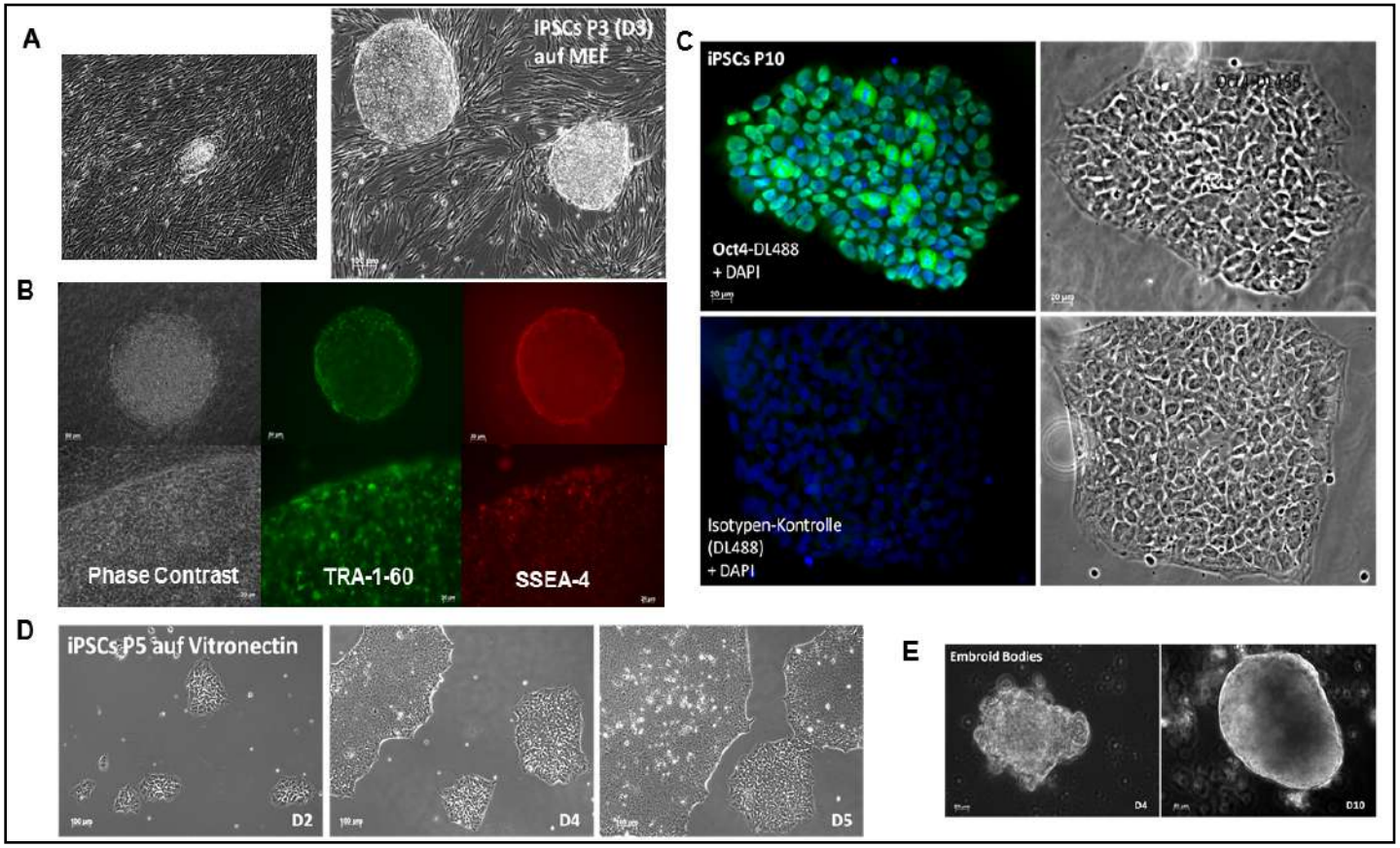
Transfection of cells with modified synthetic mRNA



Avci-Adali M., et al. (2014) *J Biol Eng.* 8(1):8

Generation of iPSCs

→ Treatment of human fibroblasts with Yamanaka factors (Oct4, Klf4, cMyc, Lin28, and Sox2) encoding mRNAs for the generation of iPSCs



Disadvantages of synthetic mRNA-based method

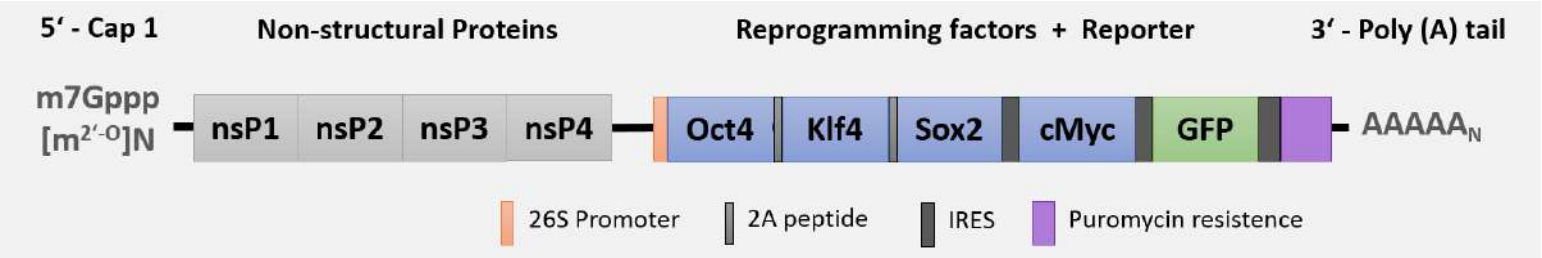
→ Daily transfection / treatment

→ Expensive and time-consuming

→ Low efficiency

Self-replicating mRNA (srRNA)

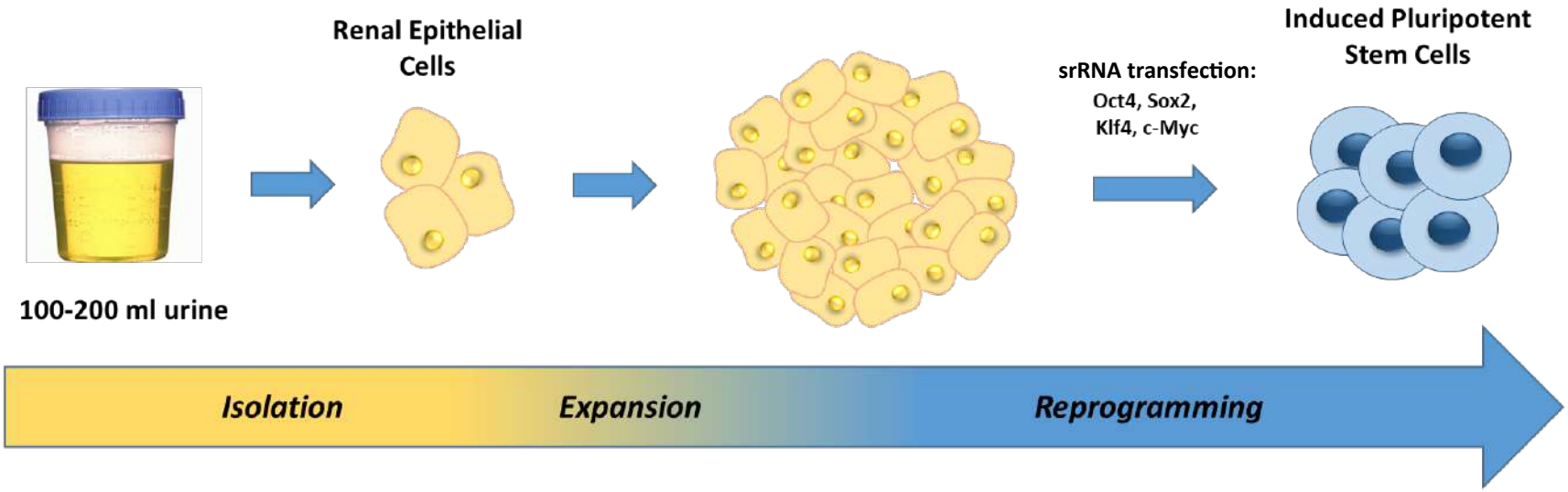
VEE (Venezuelan equine encephalitis)



~ 15 kb

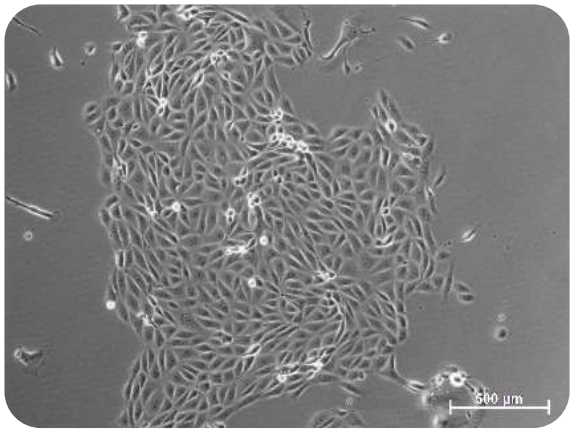
Steinle H, …, ***& Avci-Adali M.*** Stem Cells Int. (2019) 2019:7641767
*Umrath F,…, **& Avci-Adali M.** Int J Mol Sci. (2019), 20(7):1648*

Reprogramming of urine-derived renal epithelial cells into iPSCs



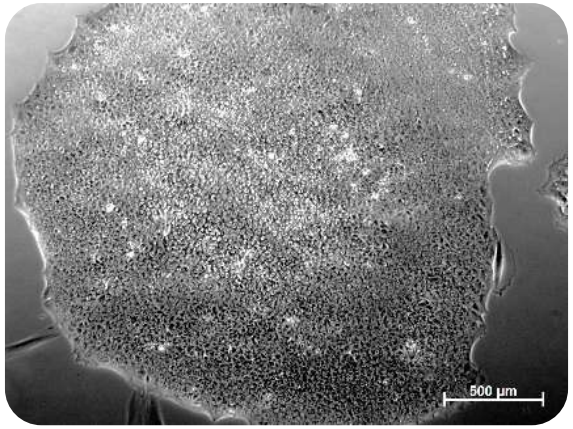
Reprogramming of urine-derived renal epithelial cells into iPSCs

Human renal epithelial cells

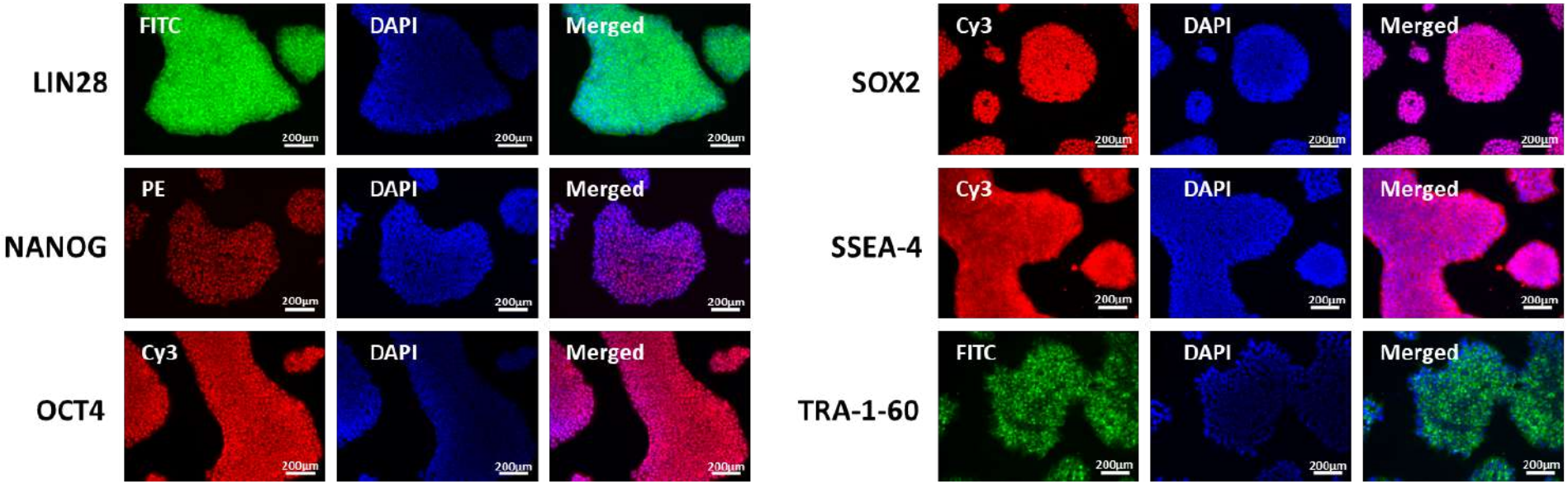


srRNA

iPSCs

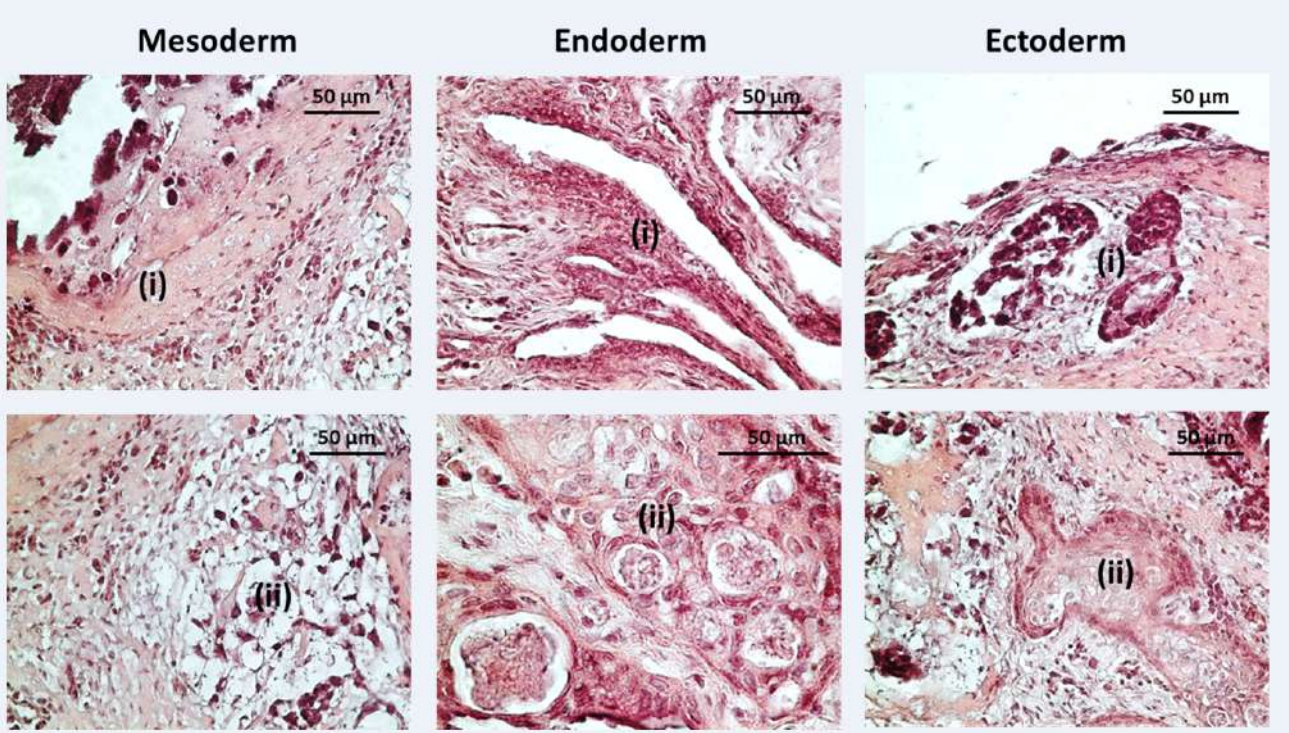


Reprogramming of urine-derived renal epithelial cells into iPSCs



Steinle, H.,… **Avci-Adali, M.** 2019. *Molecular Therapy-Nucleic Acids*, *17*, 907-921.

Reprogramming of urine-derived renal epithelial cells into iPSCs



Steinle, H.,… **Avci-Adali, M.** 2019. *Molecular Therapy-Nucleic Acids*, *17*, 907-921.

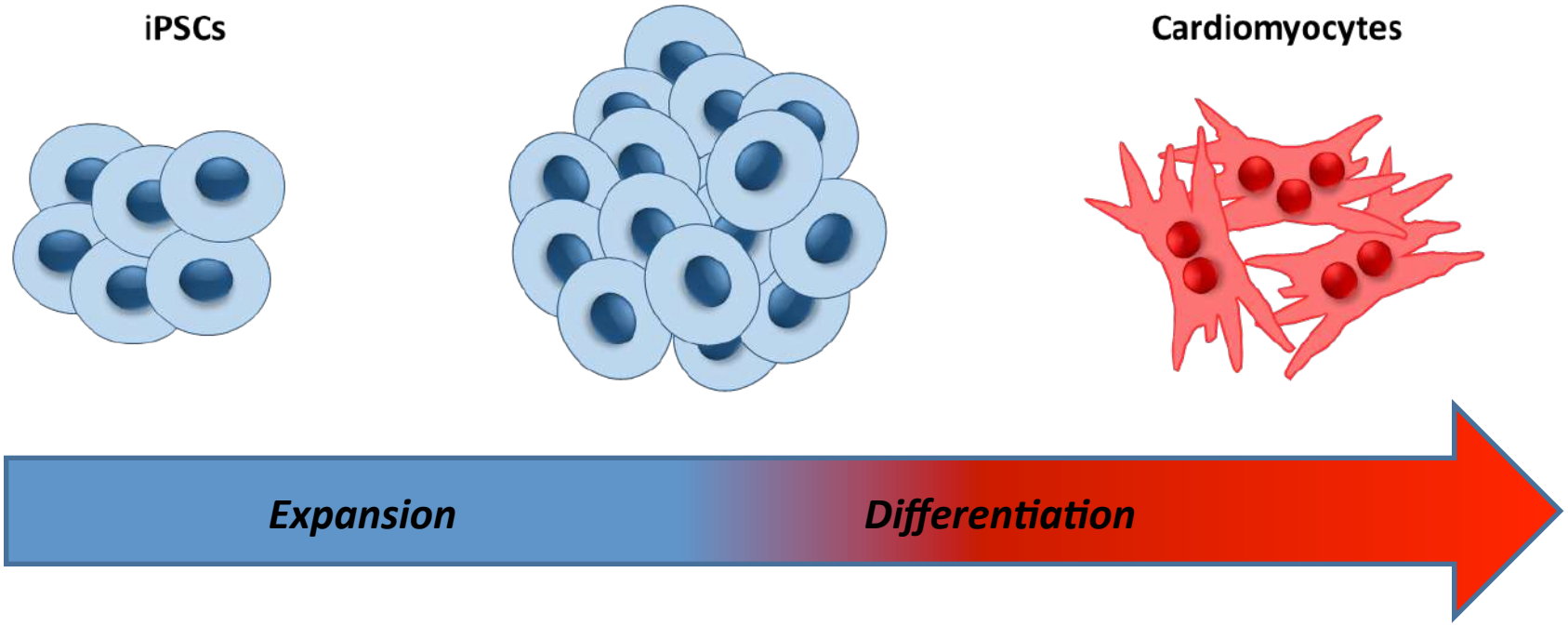
Self-replicating mRNA (srRNA)

→ Only one single transfection is required

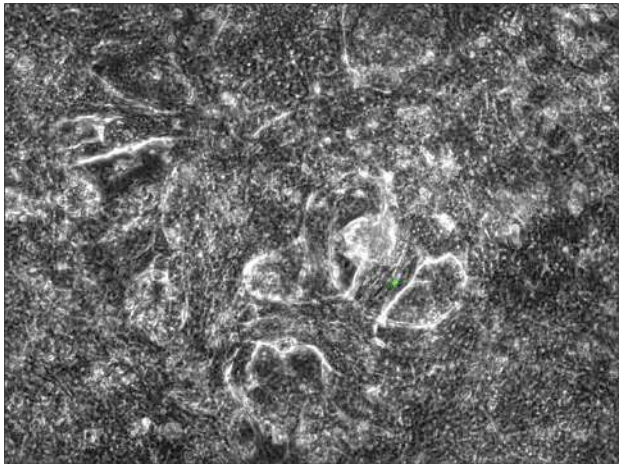
→ Higher reprogramming efficiency

→ No integration into host genome

Differentiation of iPSCs into cardiomyocytes



Generation of beating cardiomyocytes



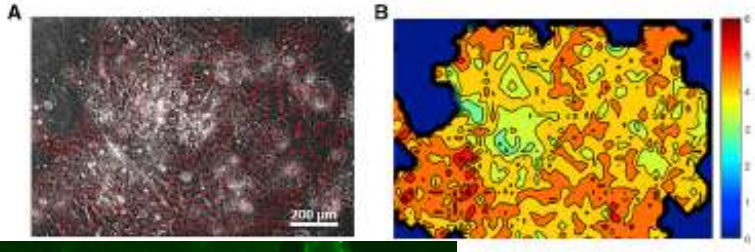
Molecular Therapy
Nucleic Acids
Original Article

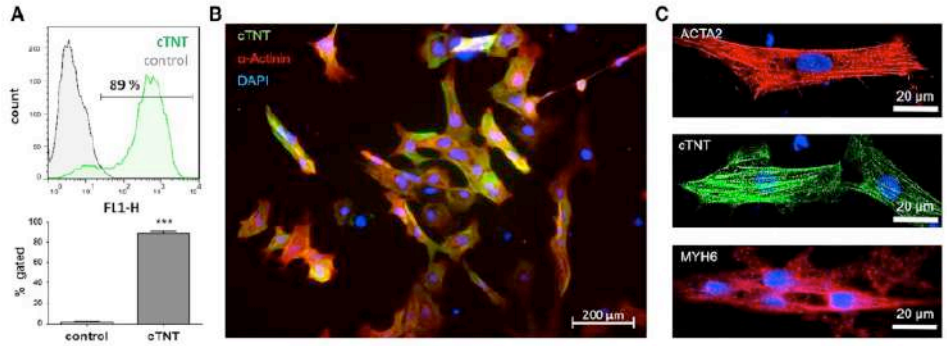
AMERICAN SOCIETY of GENE & CELL THERAPY

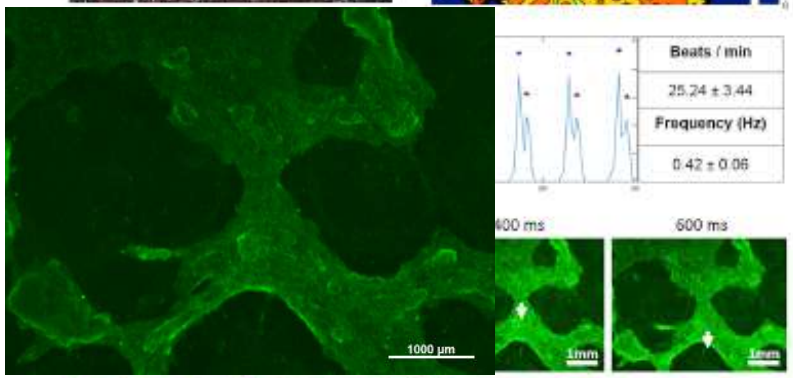
Reprogramming of Urine-Derived Renal Epithelial Cells into iPSCs Using srRNA and Consecutive Differentiation into Beating Cardiomyocytes

Heidrun Steinle,[1,4] Marbod Weber,[1,4] Andreas Behring,[1] Ulrike Mau-Holzmann,[2] Christiane von Ohle,[3] Aron-Frederik Popov,[1] Christian Schlensak,[1] Hans Peter Wendel,[1] and Meltem Avci-Adali[1]

[1]Department of Thoracic and Cardiovascular Surgery, University Hospital Tübingen, Calwerstraße 7/1, 72076 Tübingen, Germany; [2]Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen, Calwerstraße 7, 72076 Tübingen, Germany; [3]Department of Conservative Dentistry and Periodontology, Centre of Dentistry, Oral Medicine and Maxillofacial Surgery, University Hospital Tübingen, Osianderstraße 2-8, 72076 Tübingen, Germany



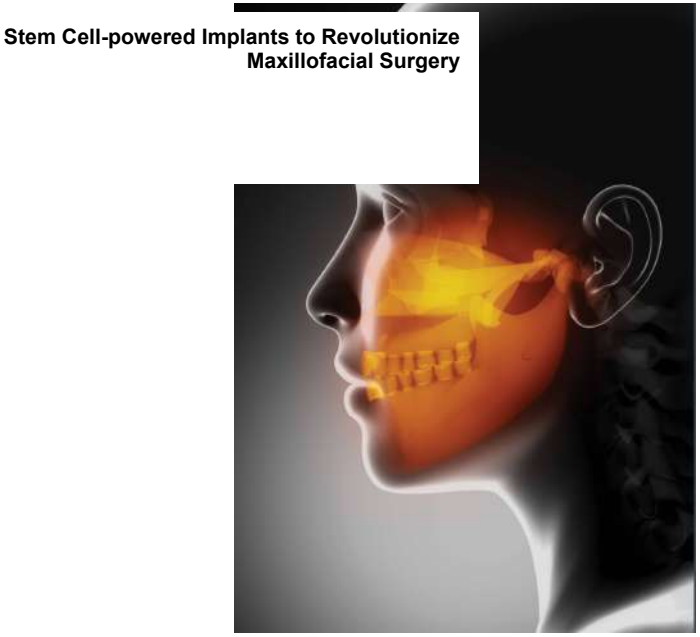


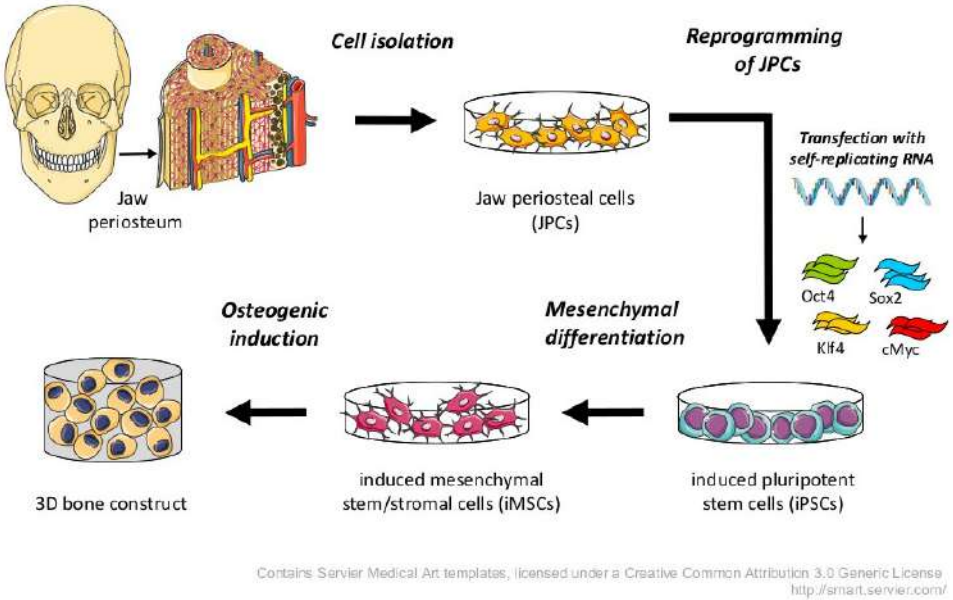


Generation of autologous iPSCs for bone regeneration

Dept. of Oral and Maxilofacial Surgery

DFG Deutsche Forschungsgemeinschaft





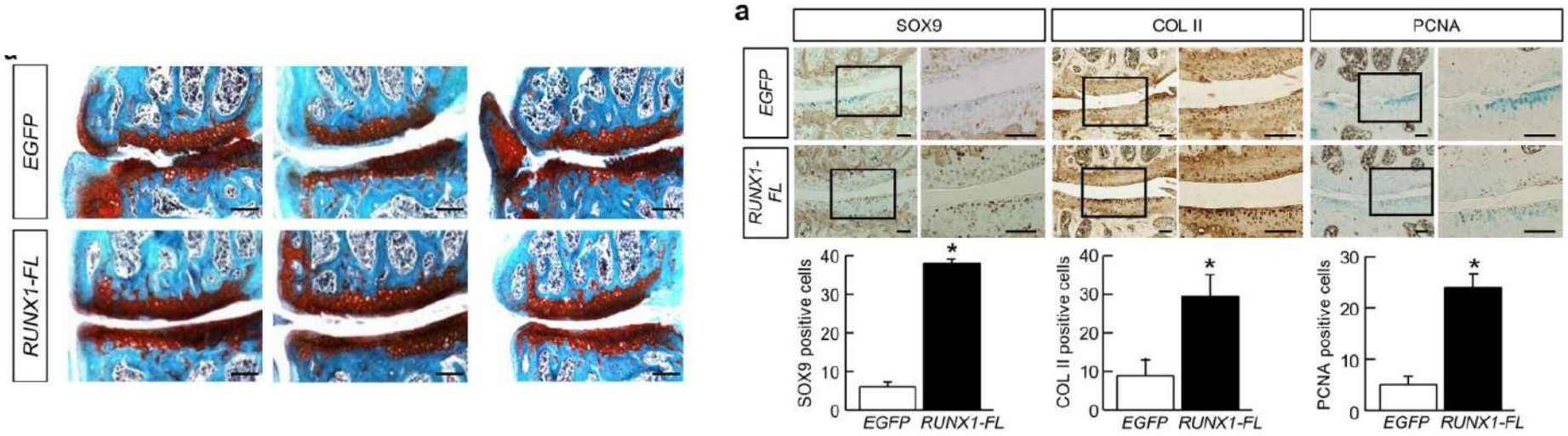
iPSC generation from jaw periosteum cells (JPCs) for bone tissue engineering

Umrath F,…, ***& Avci-Adali M.*** *Int J Mol Sci. (2019), 20(7):1648*

mRNA delivery of a cartilage-anabolic transcription factor as a disease-modifying strategy for osteoarthritis treatment

→ Polyplex nanomicelles containing cartilage-anabolic transcription factor RUNX1 mRNA

proliferating nuclear antigen (PCNA)

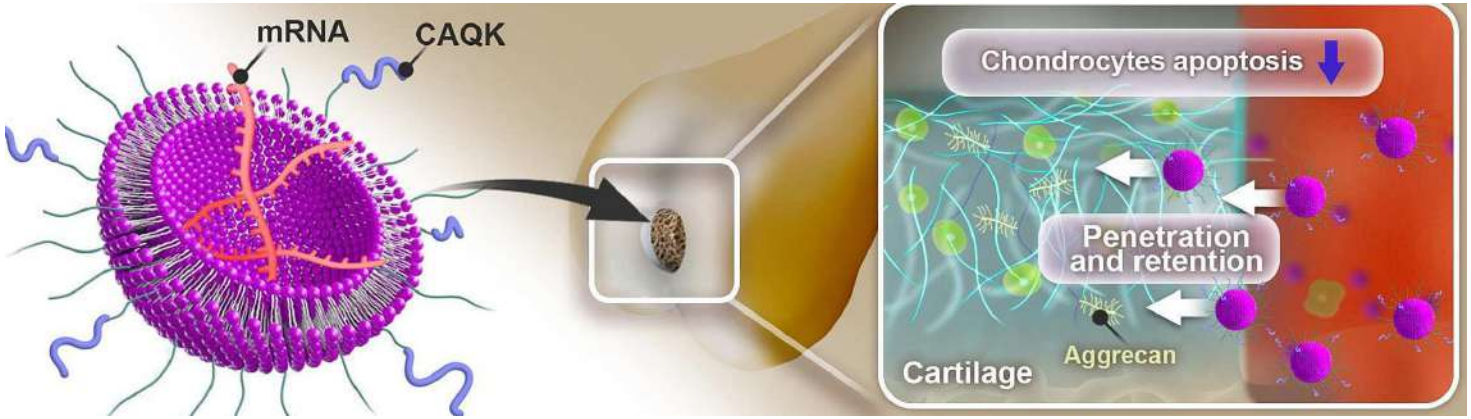


Aini H, et al. Scientific reports. 2016, 6(1):18743.

→ Delivery of RUNX1 mRNA successfully suppressed the progression of OA in mouse knee joints compared with non-treated group

→ Expression of cartilage-anabolic and proliferation markers was increased in articular chondrocytes of the RUNX1 mRNA injected knees

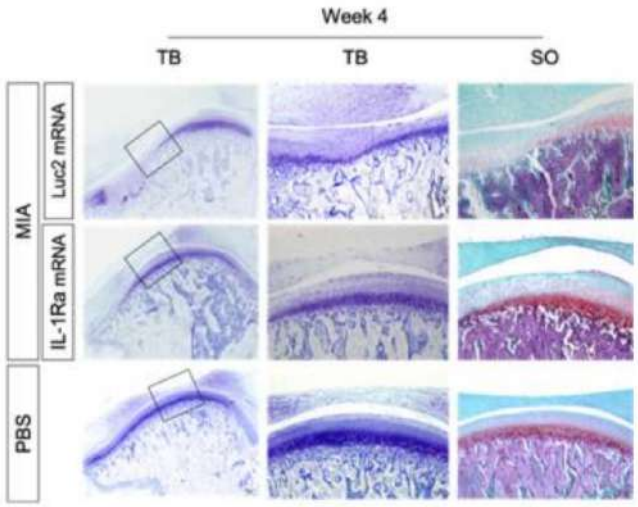
Cartilage-targeting mRNA-lipid nanoparticles rescue perifocal apoptotic chondrocytes for integrative cartilage repair



Yu X, et al. Chemical Engineering Journal. 2023;465:142841.

→ IGF-1 mRNA was encapsulated into ionizable lipid nanoparticles (LNPs)
→ CAQK peptide modification of LNPs led to improved penetration of cartilage and prolonged retention in the joint cavity
→ IGF-1 mRNA loaded LNPs showed robust reversal of chondrocyte apoptosis
→ In a full-thickness chondral defect model, IGF-1 mRNA loaded LNPs maintained interfacial cellularity and prevented matrix degradation.

Anti-Inflammatory Therapy for Temporomandibular Joint Osteoarthritis Using mRNA Medicine Encoding Interleukin-1 Receptor Antagonist



toluidine blue (TB), safranin-O (SO)

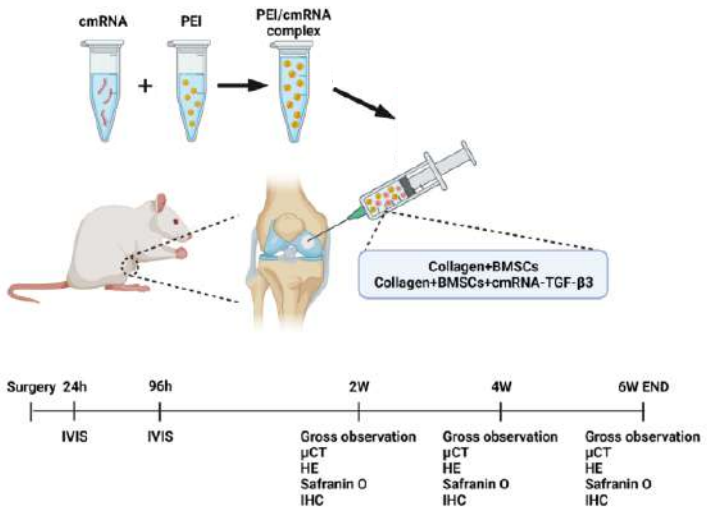
Deng J, et al. Pharmaceutics. 2022;14(9):1785.

The temporomandibular joint (TMJ) OA causes long-lasting joint pain with chronic inflammation.

→To develop an anti-inflammatory therapy, interleukin-1 receptor antagonist (IL-1Ra) encoding mRNA loaded polyplex nanomicelles were injected into the rat model of the TMJs

→A single administration of 2.5 μg of IL-1Ra mRNA provided sustained pain relief and an inhibitory effect on OA progression for 4 weeks.

Highly efficient healing of critical sized articular cartilage defect in situ using a chemically nucleoside-modified mRNA-enhanced cell therapy

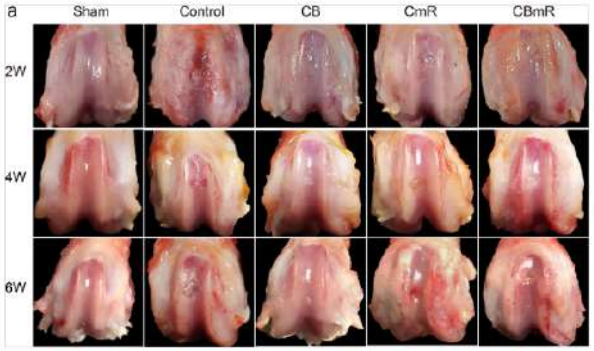


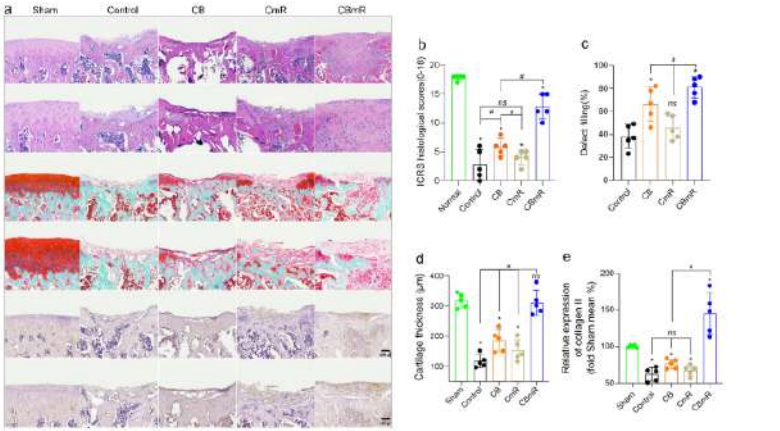
Zhong G, et al. bioRxiv. 2022, 2022-05.

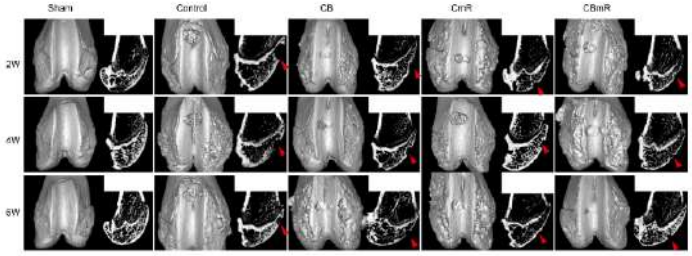
TGF-β3 plays a key role in cartilage regeneration and it can induce chondrogenic differentiation of MSCs and promote cartilage-like matrix deposition.

→ Single injection of collagen I containing BMSCs without or with 20 μg modified TGF-β3 mRNA into the critical-sized cartilage defects of rats

Highly efficient healing of critical sized articular cartilage defect in situ using a chemically nucleoside-modified mRNA-enhanced cell therapy







Zhong G, et al. bioRxiv. 2022, 2022-05.

Sham group: without cartilage defect
Control group: cartilage defect creation and injection of PBS
CB group: injection of Collagen I and BMSCs mixture
CmR group: injection of Collagen I and TGFβ3 mRNA
CBmR group: injection of Collagen I, BMSCs ,and TGFβ3 mRNA

→ Group injected with collagen I, BMSCs ,and TGFβ3 mRNA showed a smooth joint surface and improved cartilage regeneration after 4 and 6 weeks

→ Compared to the group without TGF-β3 mRNA reduced subchondral bone abnormalities increased cartilage thickness, filling of the defect, and an increase in type II collagen were detected.

→ µCT analyses showed that TGF-β3 mRNA not only promoted cartilage regeneration but also inhibited the pathological changes of subchondral bone

Conclusion

Synthetic mRNA technology offers several potential application possibilities for the treatment of osteoarthritis:

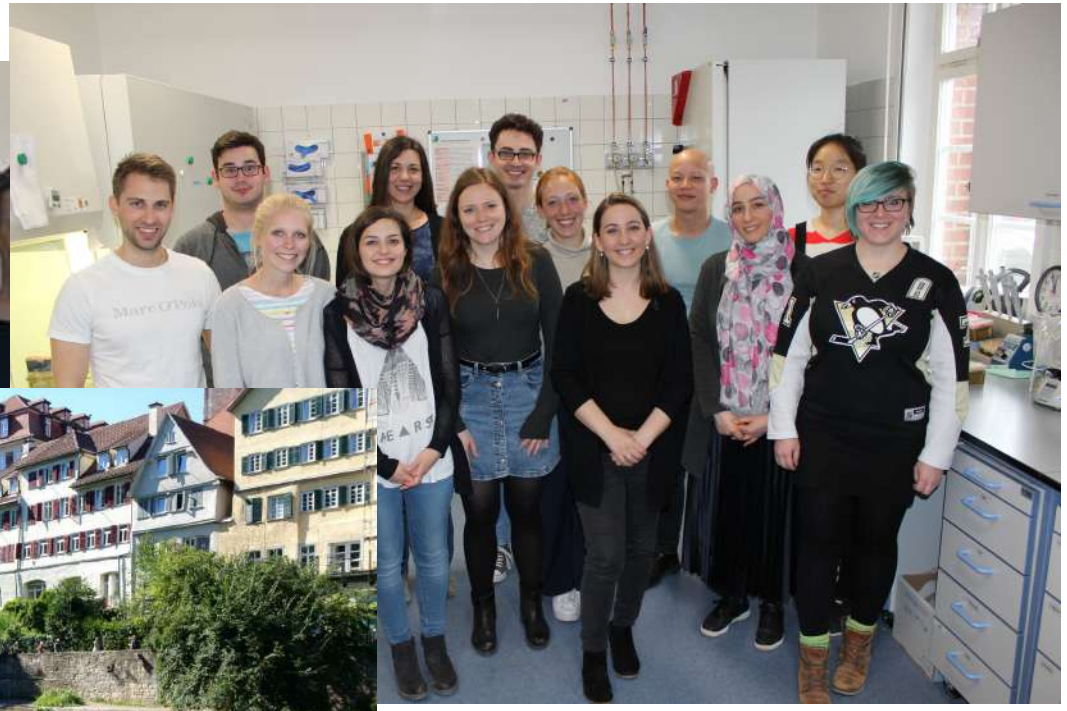
→ Disease-Modifying Osteoarthritis Drugs
- Synthetic mRNAs encoding proteins that promote the synthesis of ECM components or inhibit cartilage-degrading enzymes to prevent the progression of OA

→ Pain Management
- Synthetic mRNAs encoding proteins for pain relief

→ Growth Factors
- Stimulate the production of new cartilage tissue and enhance the healing process

→ Prevention of inflammation
- Synthetic mRNAs encoding anti-inflammatory proteins to reduce inflammation and slow down the progression of the disease

Thank you for your attention!



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Universitätsklinikum
Tübingen

Combination of synthetic mRNA with implants

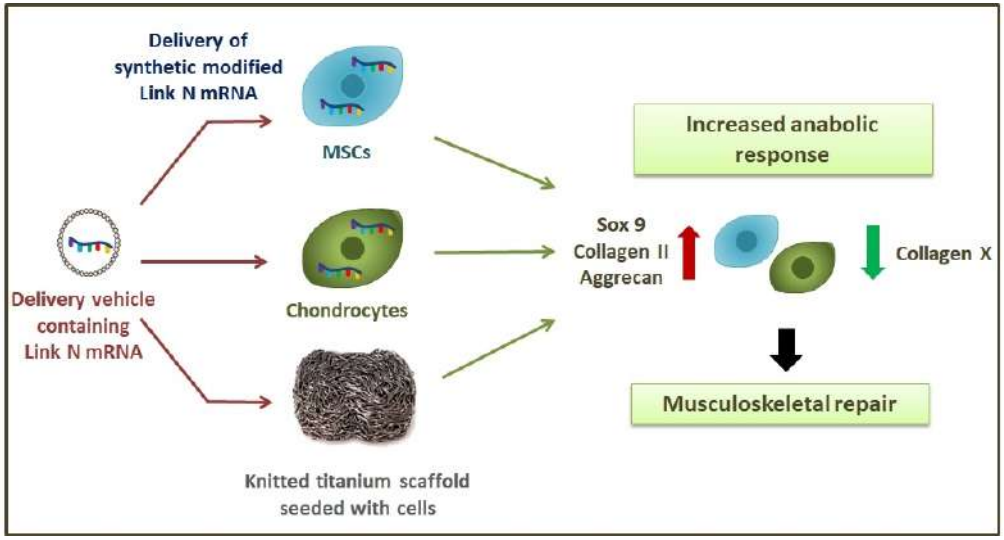
International Journal of *Molecular Sciences*

MDPI

Article

Exogenous Delivery of Link N mRNA into Chondrocytes and MSCs—The Potential Role in Increasing Anabolic Response

Gauri Tendulkar [1,*], Sabrina Ehnert [1], Vrinda Sreekumar [1], Tao Chen [1], Hans-Peter Kaps [1], Sonia Golombek [2], Hans-Peter Wendel [2], Andreas K. Nüssler [1] and Meltem Avci-Adali [2]



BG Kliniken

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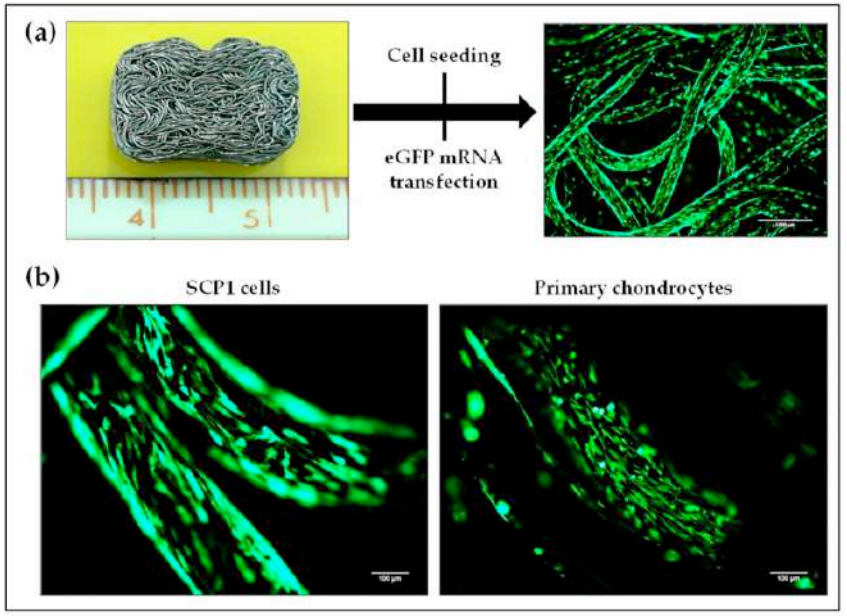
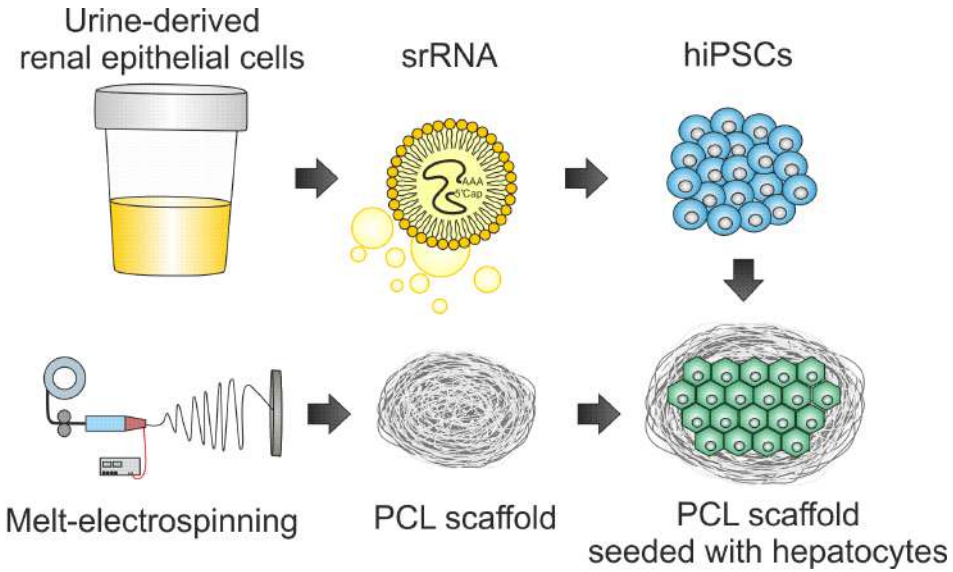
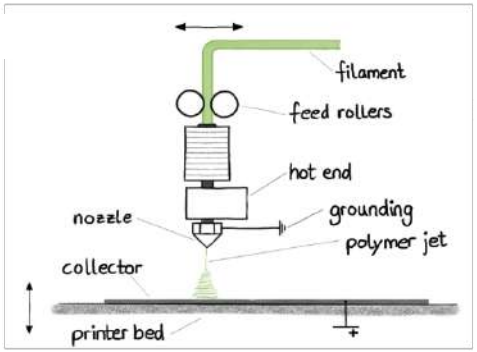
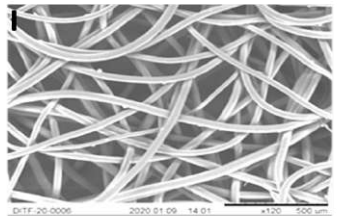
Siegfried Weller Institute for Trauma Research

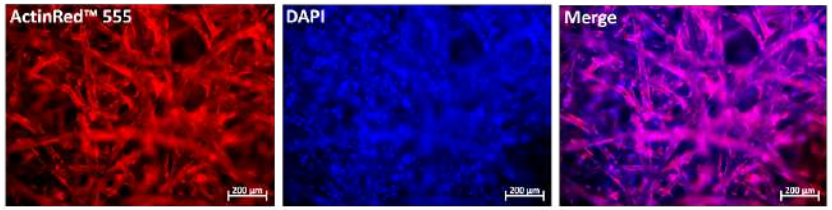


Footprint-free generation of autologous hepatocytes









Possible application in liver tissue engineering and drug testing

DEUTSCHE INSTITUTE FÜR
TEXTIL+FASERFORSCHUNG

Innovative aspects of the strategy

✓**Self-replicating mRNA is degradable and not genome-integrating**
→ No induction of mutagenesis
→ Generation of clinically applicable cells

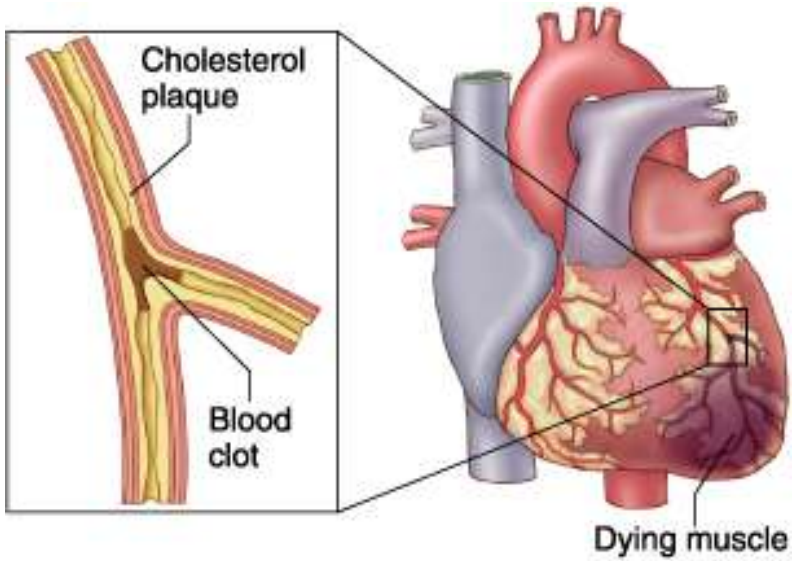
✓ **Non-invasive collection of patient cells** from urine
→ Destruction of healthy tissue not necessary

✓ **Generation of desired cells from patient's own somatic cells**
→ autologous cells
→ no rejection reactions
→ personalized treatment

✓ **Regeneration of tissues**

Acute myocardial infarction - loss of cardiomyocytes

Heart attack #1 cause of death

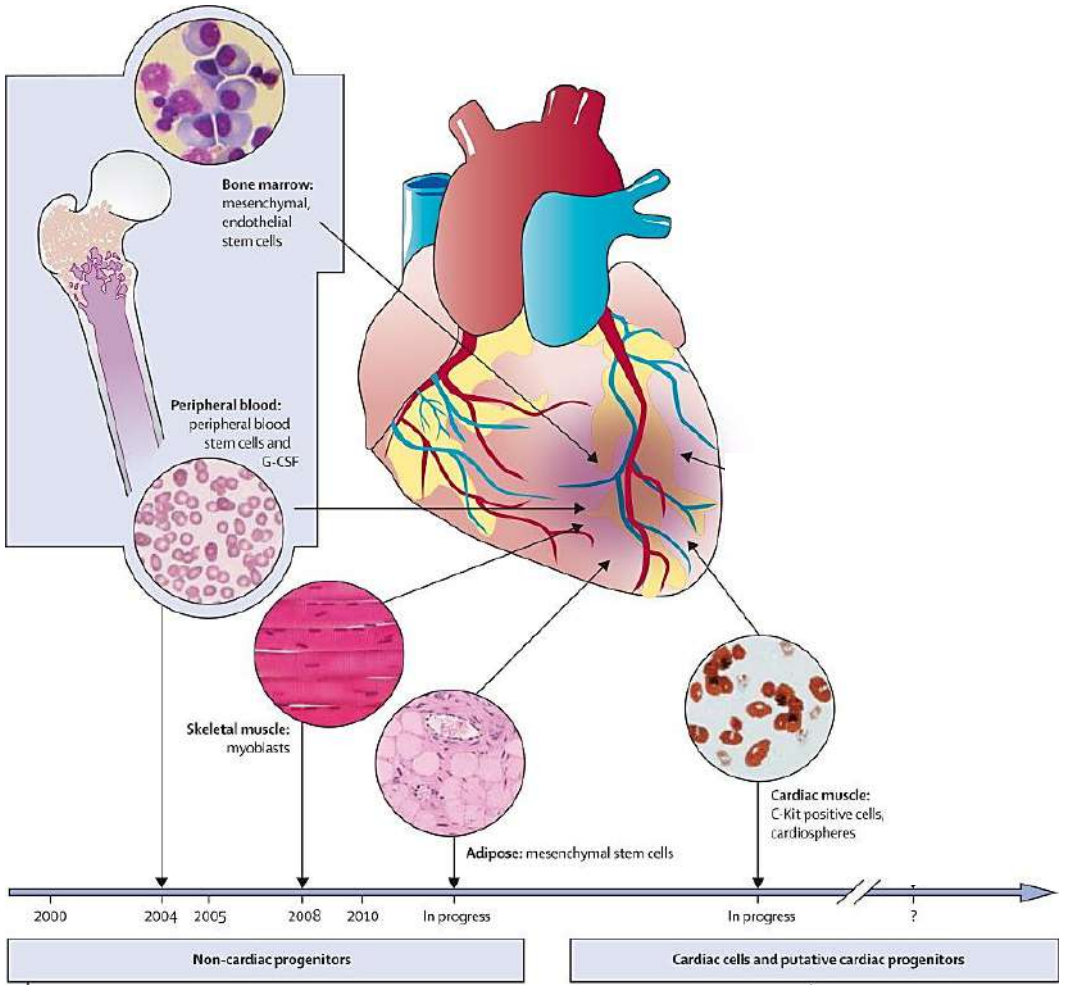


http://www.mdguidelines.com/myocardial-infarction-acute

- Death of cardiomyocytes
- Very low proliferation ability of adult cardiomyocytes
- Scar tissue replacement

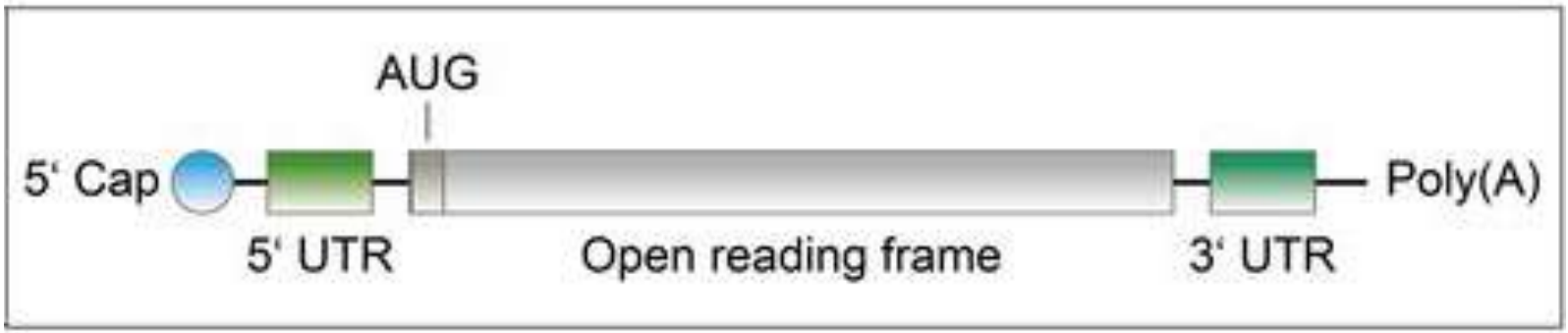
Impaired heart function

Therapy approaches for the regeneration of the heart muscle



Modifiziert nach Ptaszek L.M., et al., Lancet (2012) 379 (9819): 933-42

Modified synthetic messenger RNA (mRNA)

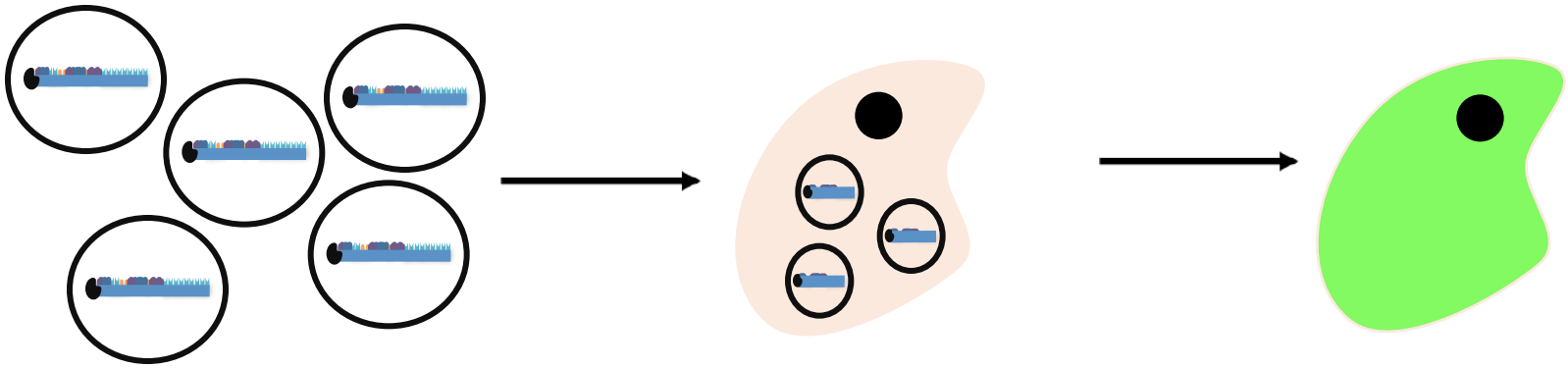


Beck JD, et al. mRNA therapeutics in cancer immunotherapy. Molecular cancer. 2021, 20(1):1-24.

Implantable cells

Cells	Advantages	Disadvantages
Skeletal myoblasts	Easy to isolate High proliferation rate Hypoxia-resistant Autologous	Incidence of cardiac arrhythmias
Stem cells from bone marrow	Easy to isolate Multipotent Low immune responses Autologous	Limited availability Bone and cartilage generation in myocardium
Stem cells from adipose tissue	Easy to isolate High Availability Multipotent Low immune responses	Low survival
Kardiale Stammzellen	Multipotent Autolog	Limited availability

Transfection of cells with modified synthetic mRNA



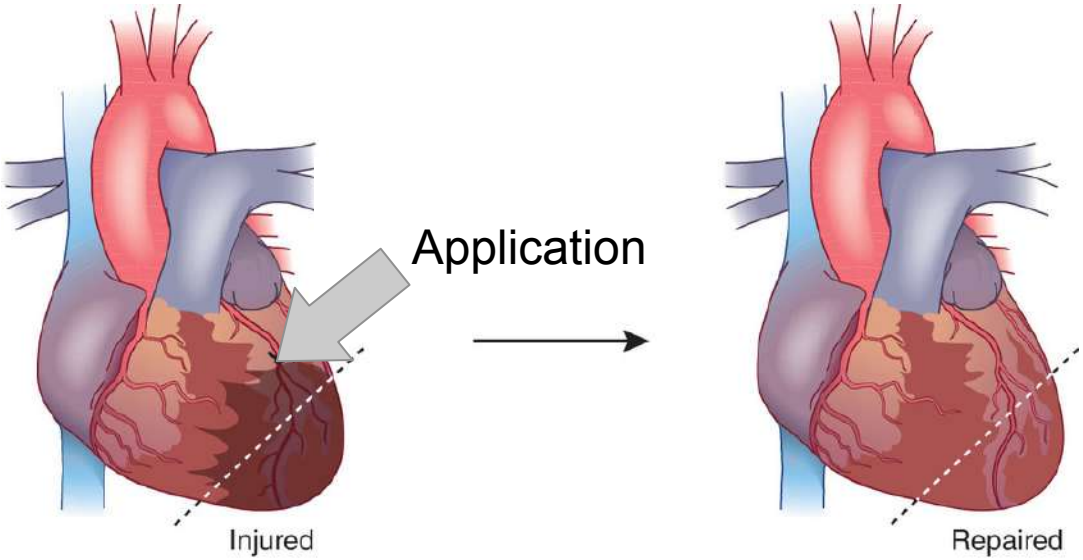
Generation of lipoplexes for mRNA transfection

mRNA transfected cell

eGFP positive cell

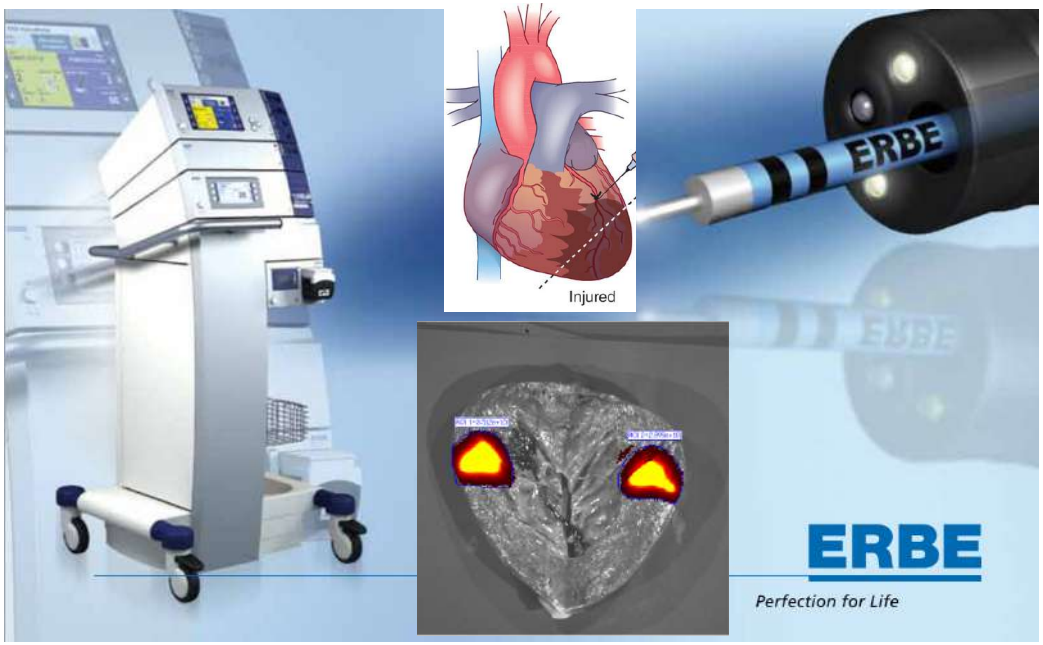
Application of cardiomyocytes generated from iPSCs into the myocardium

How can we deliver these cardiomyocytes into the myocardium?



http://www.nature.com/nm/journal/v19/n4/images/nm.3147-F1.jpg

Application of cardiomyocytes generated from iPSCs into the myocardium



XenoLight DiR fluorescent dye

www.nature.com/scientificreports

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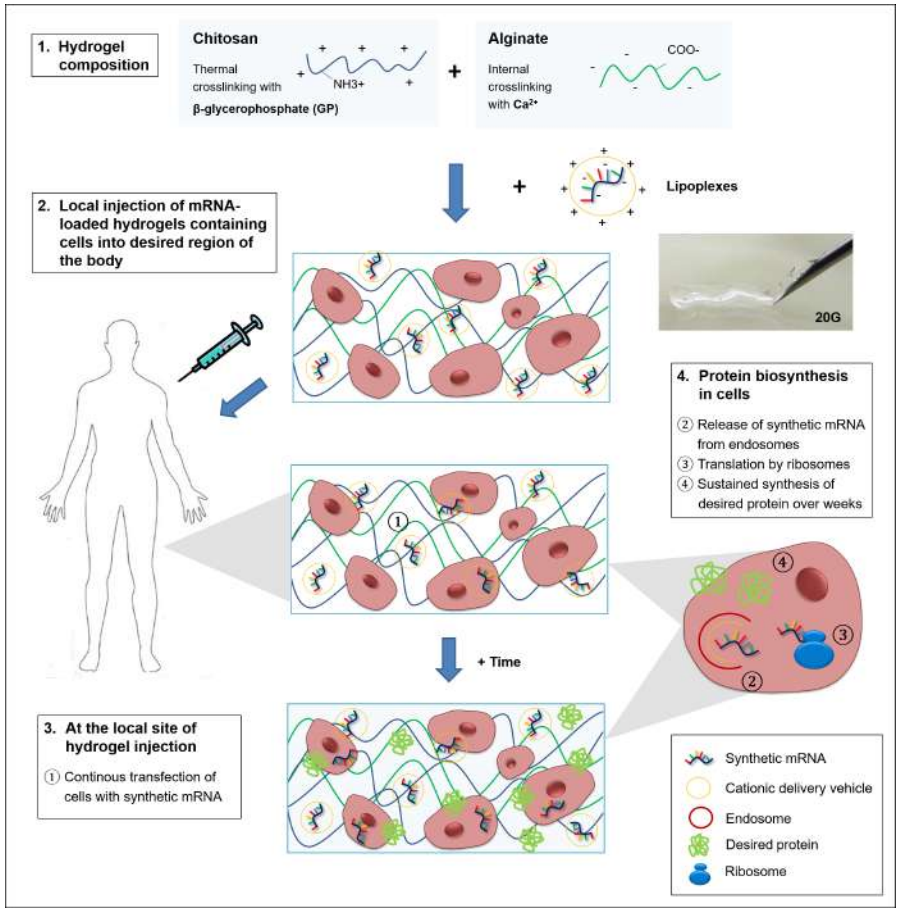
Hydrojet-based delivery of footprint-free iPSC-derived cardiomyocytes into porcine myocardium

Marbod Weber[1], Andreas Fech[2], Luise Jäger[2], Heidrun Steinle[1], Louisa Bühler[2], Regine Mariette Perl[3], Petros Martirosian[3], Roman Mehling[4], Dominik Sonanini[4], Wilhelm K. Aicher[5], Konstantin Nikolaou[3], Christian Schlensak[1], Markus D. Enderle[2], Hans Peter Wendel[1], Walter Linzenbold[2] & Meltem Avci-Adali[1]

mRNA releasing hydrogels



International Journal of *Molecular Sciences*

MDPI

Article

Incorporation of Synthetic mRNA in Injectable Chitosan-Alginate Hybrid Hydrogels for Local and Sustained Expression of Exogenous Proteins in Cells

Heidrun Steinle, Tudor-Mihai Ionescu, Selina Schenk, Sonia Golombek, Silju-John Kunnakattu, Melek Tutku Özbek, Christian Schlensak, Hans Peter Wendel and Meltem Avci-Adali *

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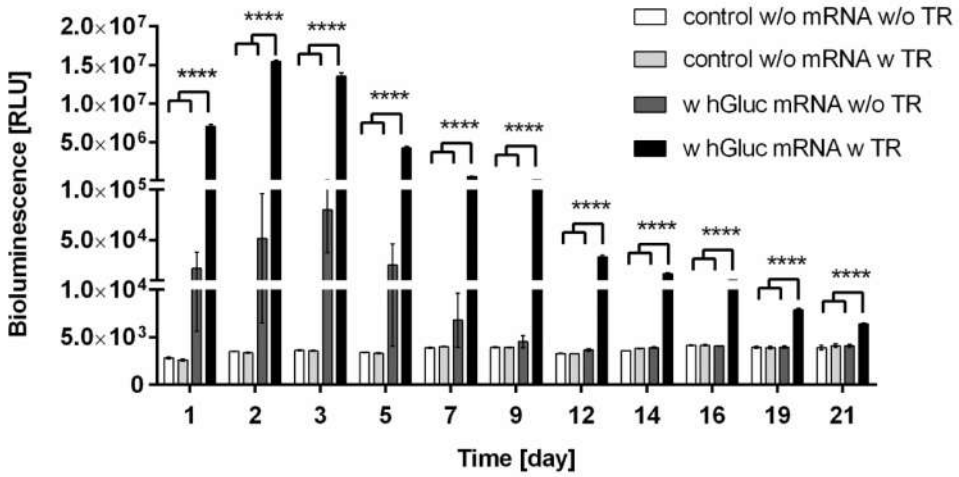
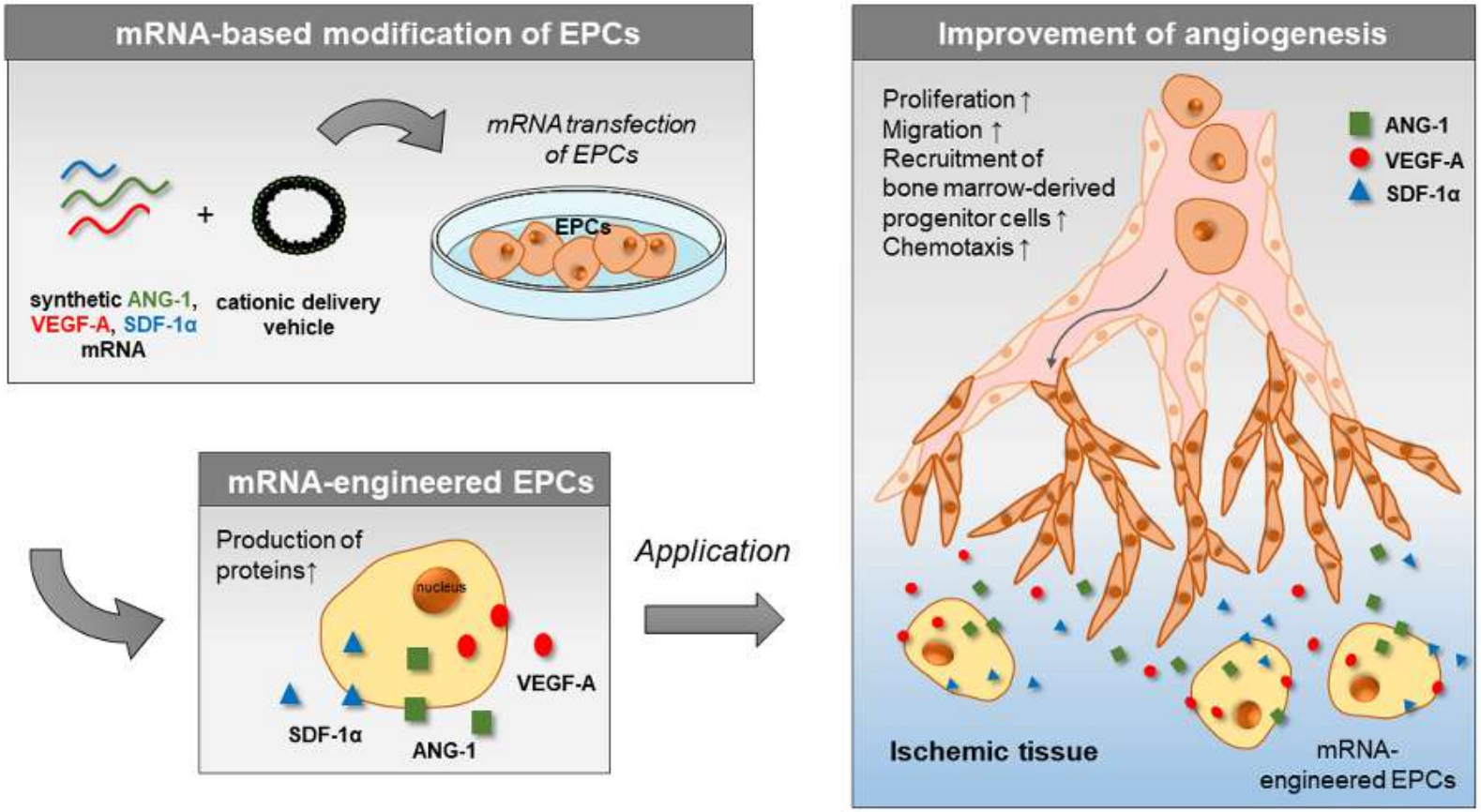


Steinle H., …, ***Avci-Adali M.*** *Int. J. Mol. Sci.* **2018**, *19*(5)

Modification of endothelial progenitor cells (EPCs) using synthetic mRNA



Anwendung von synthetischer mRNA zur Modifikation von Zellen

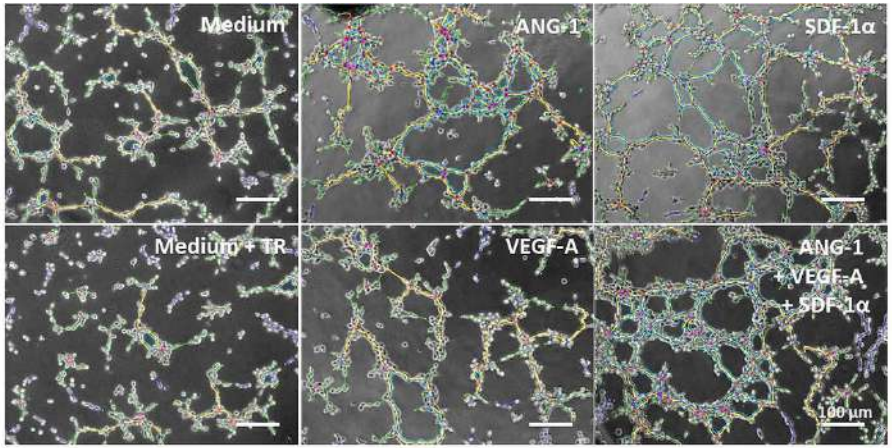
Molecular Therapy
Nucleic Acids
Volume 13, 7 December 2018, Pages 387-398
open access

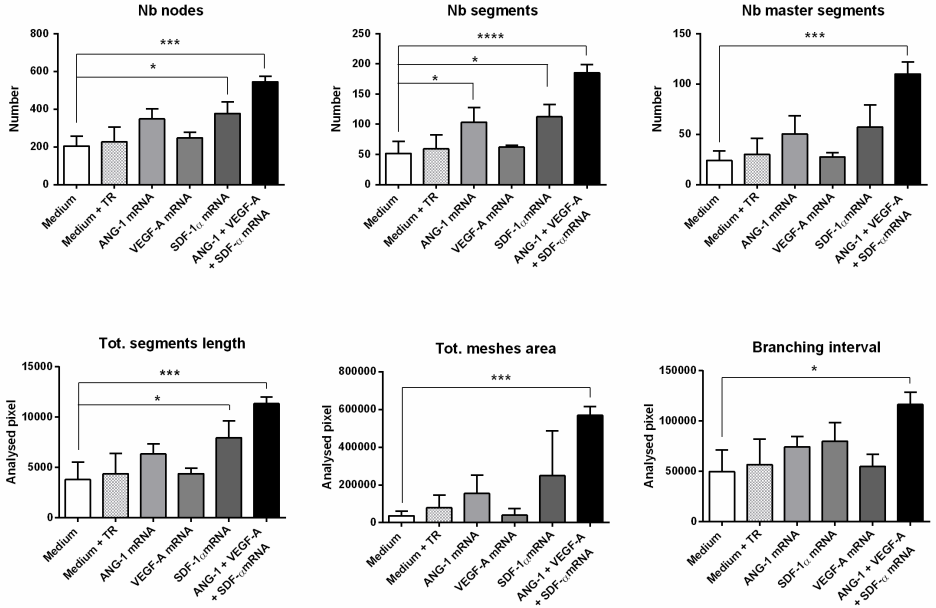
Original Article

Improving the Angiogenic Potential of EPCs via Engineering with Synthetic Modified mRNAs

Heidrun Steinle [1], Sonia Golombek [1], Andreas Behring [1], Christian Schlensak [1], Hans Peter Wendel [1], Meltem Avci-Adali [1]

Tube formation assay





Modification of endothelial progenitor cells (EPCs) using synthetic mRNA

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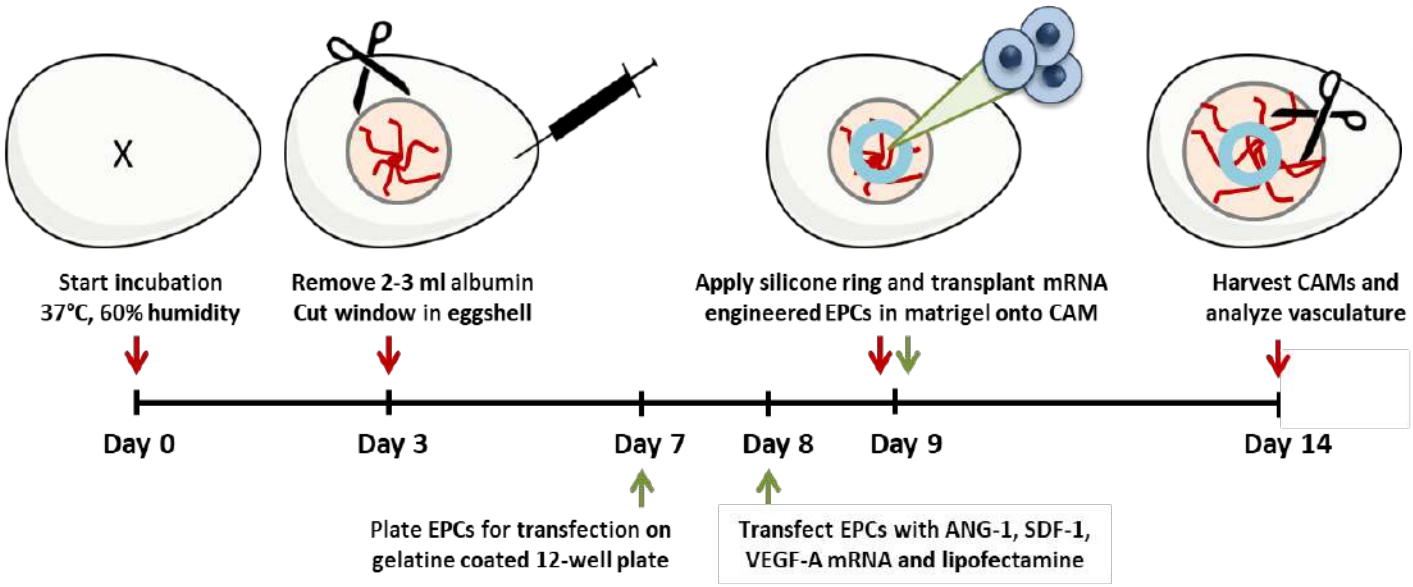
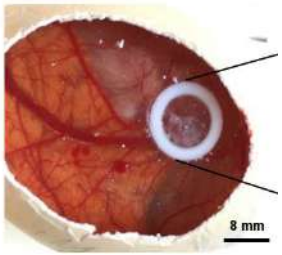
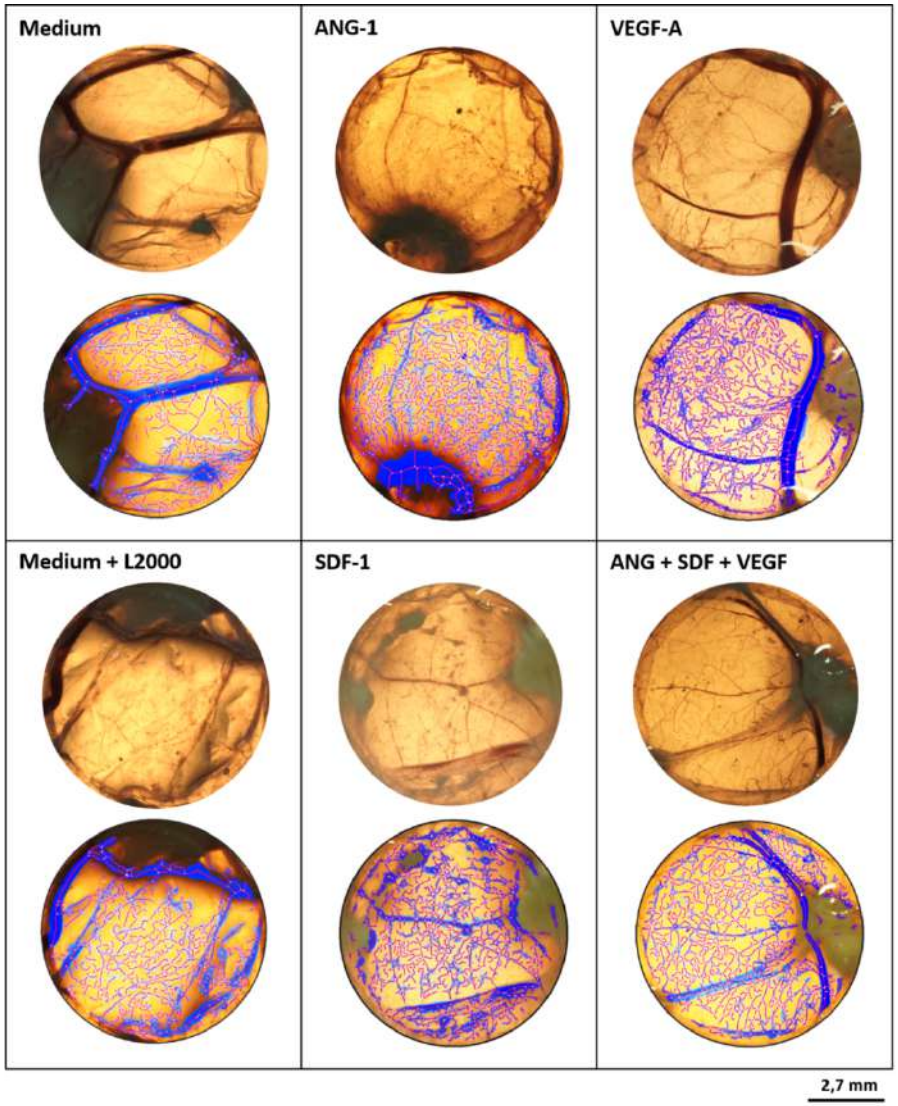
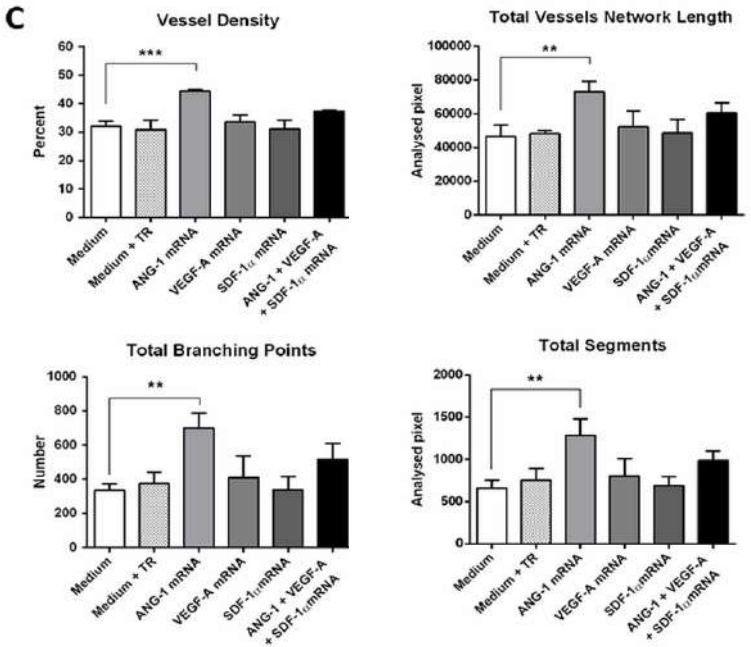
Chorion-Allantois-Membran (CAM) assay





Modification of endothelial progenitor cells (EPCs) using synthetic mRNA





→ ANG-1 mRNA transfected EPCs showed significantly enhanced angiogenic potential

Footprint-free generation of autologous rejuvenated skeletal myocytes for sphincter muscle repair

Dept. of Urology

DFG Deutsche Forschungsgemeinschaft

Fused myotubes