Karlsruhe Institute of Technology

Institute for Biological Interfaces
IBG-1 | Biomolecular Micro- and Nanostructures

DNA Nanocomposites as Functional Materials in Cells and in Cell-free Systems

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Institute for Biological Interfaces (IBG-1)

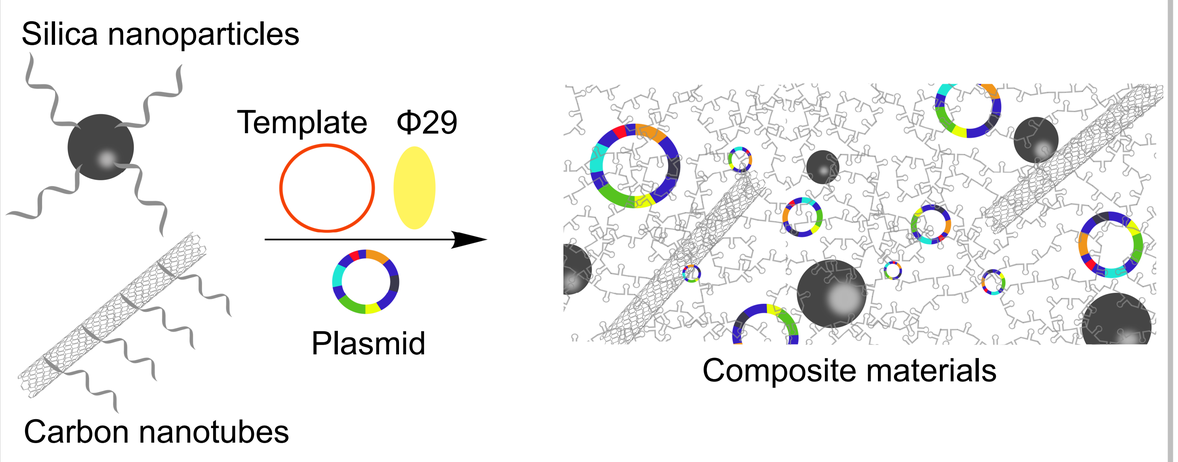
Synthesis of tailorable nanocomposites with non-linear mechanical stiffness and viscosity properties as substrate for cell culture and for cell-free protein expression

Relation to P3T3 workplan

ST 1.3 DNA Materials (programmable interfaces)
ST 2.1 Cell populations on surfaces (dynamic scaffolds)
ST 3.2 Biohybrid Systems with integrated cell-free production

Synthesis of Biohybrid Composites

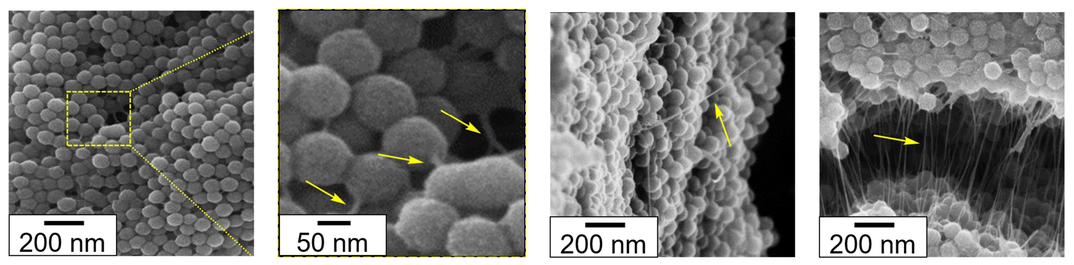
- Synthesis via **R**olling **C**ircle **A**mplification with Φ29 polymerase
- Integration of plasmid for fluorescent protein → genetically coding materials



- Adjustment of mechanical properties via SiNP/CNTs ratio
- Nomenclature is based on concentration ratio

Characterization

- **S**canning **E**lectron **M**icroscopy[1]



- **M**ultiple **P**article **T**racking Microrheology[1]

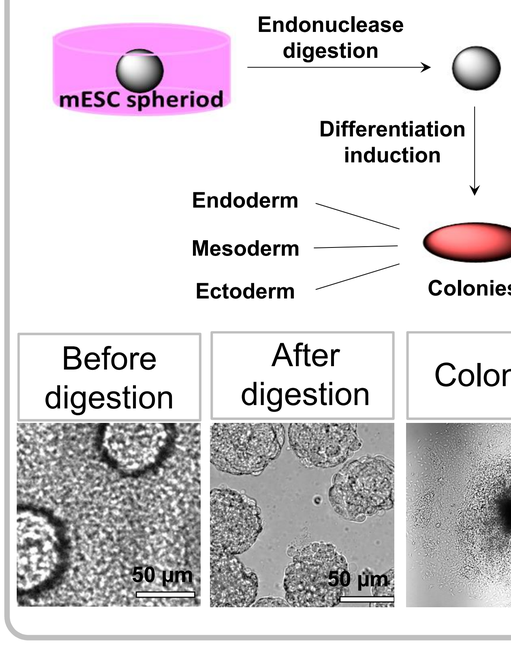
Composite material	Storage modulus G_0 [Pa]
S100	3,2 ± 0,4
SC50	4,8 ± 0,2
SC25	8,5 ± 0,7
C100	2,8 ± 0,2

Higher ratio of SiNP/CNTS ➡ stiffer material

Areas of application in eukaryotic cell culture & protein expression

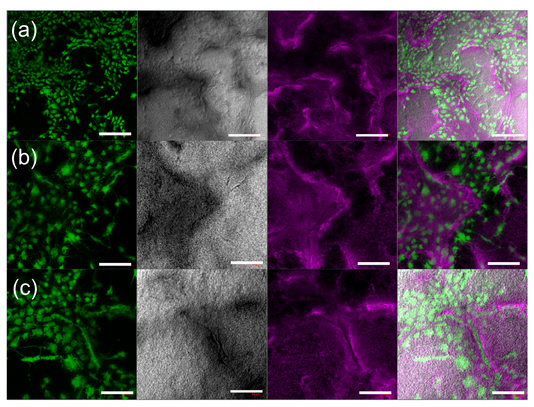
Cultivation of mESC spheroids[1]

- Nanocomposites allow the cultivation of stem cells
- Differentiation takes place after digestion



Cultivation of Osteoblasts

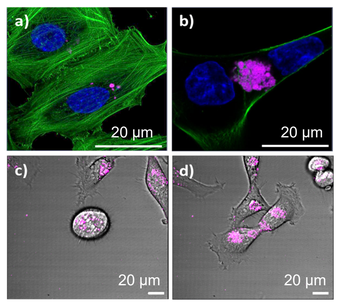
- Without differentiation medium cell morphology changes through the 3D cultivation substrate matching the one of differentiated bone cells



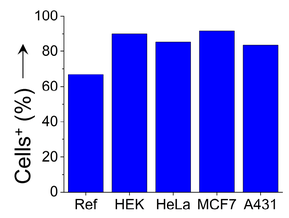
Scalebars: (a) 200 µm, (b,c) 100 µm

Uptake by various cell lines[2]

- Composites are not cytotoxic → cell division continues
- Hydrogels are ingested by different eukaryotic cell lines (>80%)



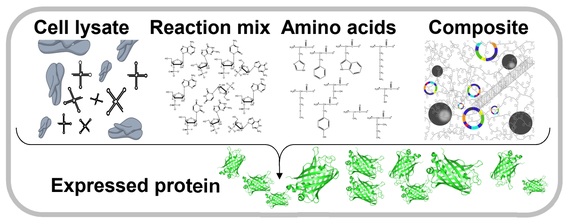
Uptake efficiency



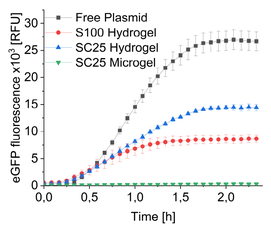
Used Cell lines:
- REF52: Rat fibroblasts (a)
- HeLa: Cervical cancer (c,d)
- HEK: Human embryonic kidney
- MCF7: Human breast cancer
- A431: Human epidermoid carcinoma (b)

Cell Free Protein Synthesis[2]

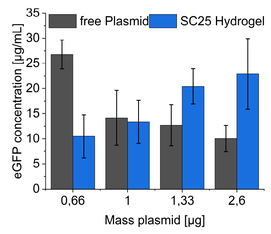
- Use of protein-coding composites with HeLa lysate



Variable materials



Variable plasmid amounts



Outlook

- Variety of biochemical and biotechnological application
- Tailored properties for cell-specific applications in cell culture
- Efficient matrix for protein synthesis in cell-free systems

Citation

[1] Y. Hu, C. M. Dominguez, J. Bauer, S. Weigel, A. Schipperges, C. Oelschlaeger, N. Willenbacher, S. Keppler, M. Bastmeyer, S. Heissler, C. Woll, T. Scharnweber, K. S. Rabe, C. M. Niemeyer, Carbon-nanotube reinforcement of DNA-silica nanocomposites yields programmable and cell-instructive biocoatings, Nat. Commun., 2019, 10, 5522.
[2] A. Schipperges, Y. Hu, S. Moench, S. Weigel, J. Reith, D. Ordoñez-Rueda, K. S. Rabe, C. M. Niemeyer, Formulation of DNA nanocomposites: Towards functional materials for protein expression, Polymers, 2021, 13, 2395.