Synthetic Biology-inspired Biohybrid Materials for Tissue Engineering and Inducible Drug Delivery

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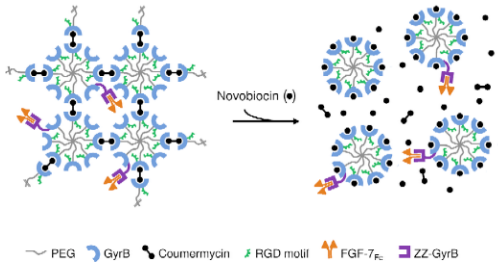
Statement of Purpose: Synthetic Biology aims at the design and implementation of biological systems with desired properties using a modular approach based on well-characterized biological building blocks. Key building blocks are regulatory elements that can be used to externally control the functionality of a biological system in space and time. Recent work has shown that such control elements can be transferred from synthetic biology to material sciences for the synthesis of stimuli-sensing biohybrid materials the properties of which can be tuned by small molecules in vitro and in mouse models[1,2] for drug delivery and tissue engineering applications.

Methods: The hydrogel precursor was synthesized by coupling cysteine-functionalized GyrB (100 µg/µl) to 8-arm PEG-vinylsulfone (40 kDa, 18 µg/µl) via Michael-type reaction. This precursor was crosslinked to a hydrogel by the addition of coumermycin at a molar ratio of GyrB : coumermycin = 2:1.
For tissue engineering experiments, an RGD motif was engineered into the GyrB protein by molecular biology methodology. The growth factor FGF-7 was produced as Fc-tagged protein and incorporated into the hydrogel by a ZZ domain genetically attached to the GyrB protein.
For drug delivery, alum-adsorbed hepatitis B vaccine was physically entrapped into the hydrogels (0.5 µg/µl gel).

Results: We selected a molecular switch from the synthetic biology toolbox that has been shown to enable tight control of gene expression in mammalian cells. This switch was based on the protein GyrB that dimerizes in the presence of coumermycin and dissociates upon addition of clinically licensed novobiocin. By coupling coumermycin-dimerized GyrB to 8-arm polyethylene glycol we synthesized a hydrogel that dissolved upon addition of novobiocin.
To validate this material for tissue engineering we incorporated a cell attachment region and the growth factor FGF-7 into the hydrogel (Fig. 1a). We demonstrated that this hydrogel efficiently supported growth of gingival keratinocytes (Fig. 1b) and fibroblasts and that the trigger-induced dissolution of the gel could be used to release the cells from the scaffold and to induce their further growth by the released factor FGF-7.
For validation as remote-controlled drug delivery device we incorporated a commercial hepatitis B vaccine into the gel and applied it subcutaneously to mice together with a prime dose of the same vaccine. Administrating novobiocin by oral route on day 7 induced the release of the cargo vaccine from the gel and boosted the immune response to protective antibody titers (Fig. 2).

a



b

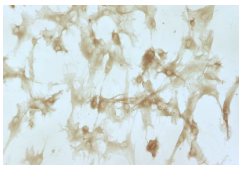


Figure 1. Design of the hydrogel and growth of gingival fibroblasts on the hydrogel.

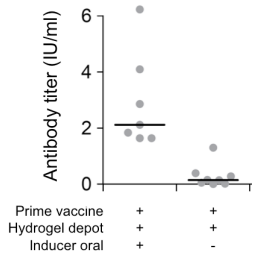


Figure 2: Immune response after induced hydrogel-mediated vaccine release 3 months post vaccination.

Conclusions: We show that molecular switches can successfully be transferred from synthetic biology to materials sciences. These switches show an inherent high degree of compatibility with a physiological environment. The induced drug release by an orally applied clinically licensed small molecule stimulus demonstrates the potential of this synthetic biology-derived approach for future developments of stimulus-controlled biohybrid materials for biomedicine.

References:

[1]Jakobus K. Chem Soc Rev 2012; 41:1000-1018
[2] Ehrbar M. Nat. Mater 2008; 7:800-804