ADVANCED FUNCTIONAL MATERIALS

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Functionalized Bioink with Optical Sensor Nanoparticles for O_2 Imaging in 3D-Bioprinted Constructs

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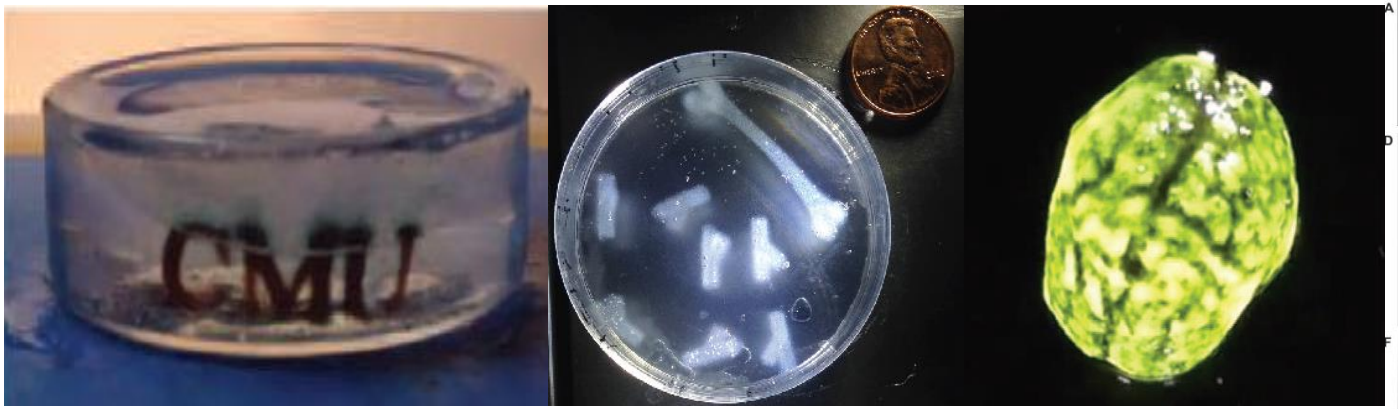
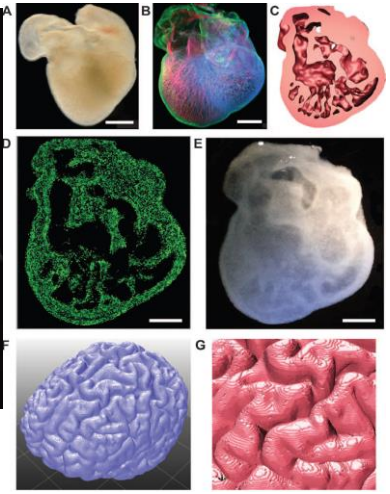
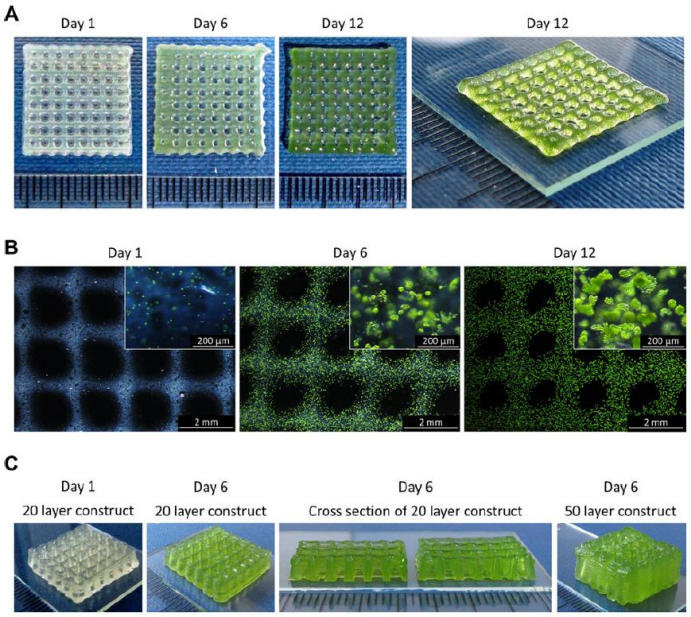
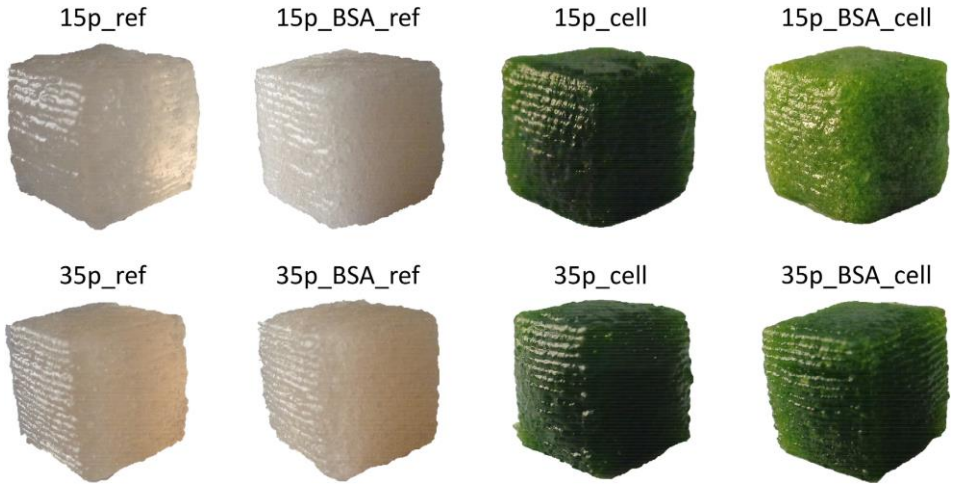
Background

Ref: Hinton et al. Sci. Adv. 2015;1:e1500758









Ref: Lode, A. *et al. Eng. Life Sci.* **15,** 177–183 (2015).

Ref: Vancauwenberghe, V.; Baiye Mfortaw Mbong, V.; Vanstreels, E.; Verboven, P.; Lammertyn, J.; Nicolai, B. *J. Food Eng.* **2017**.

In this paper

- A major limitation of current tissue engineering approaches is the insuffcient O_2 supply of 3D cell constructs due to a lack of a functional vascular system. Diffusive O2 supply into tissues is often limited to a distance of a few hundred µm and active transport to cells in deeper regions is thus important.

- There is a strong demand for novel materials and methods for mapping structural and chemical heterogeneity, for example, O_2 distribution dynamics, noninvasively in 3D-bioprinted cell constructs.

- In the present study, a focus is given on a novel sensor functionalized bioink material that enables the mapping of spatiotemporal O_2 dynamics in 3D-printed scaffolds with living cells, since O_2 is a key factor for survival and function of mammalian cells in regenerative approaches and is an important measure of metabolic activity and element cycling in biological systems in general.

3D Bioprinting with Bioink Functionalized with Sensor Nanoparticles

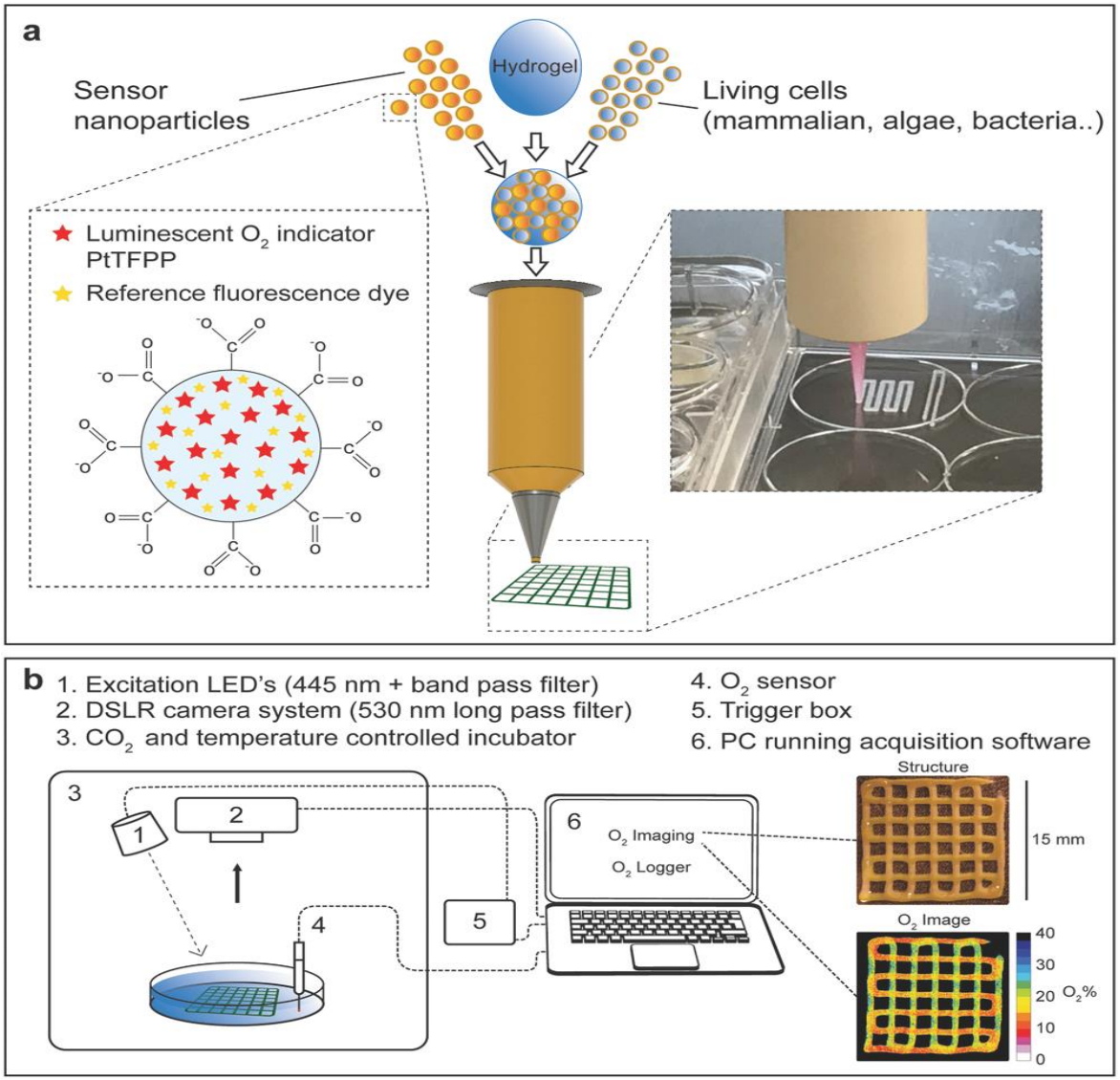


Figure 1. A novel approach for 3D bioprinting with bioink functionalized with sensor nanoparticles. a) Living cells and/or nanoparticles in hydrogel blend for extrusion based 3D bioprinting. b) Experimental setup for incubation and imaging of structure and O_2 distribution in 3D-printed hydrogel scaffolds. All components (1–4) besides the LED excitation source trigger box (5) and the PC (6) were placed inside a thermostated incubator with a controlled gas atmosphere.

Viability of Cells in Bioink with Sensor Nanoparticles

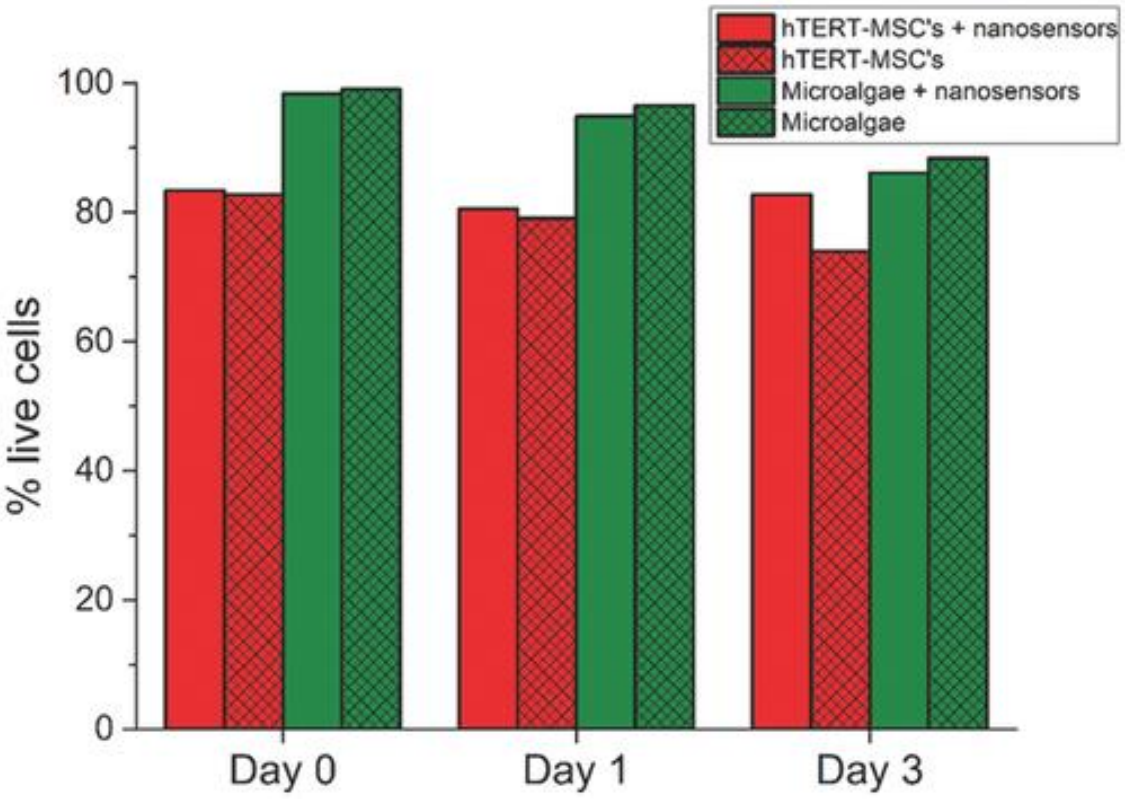
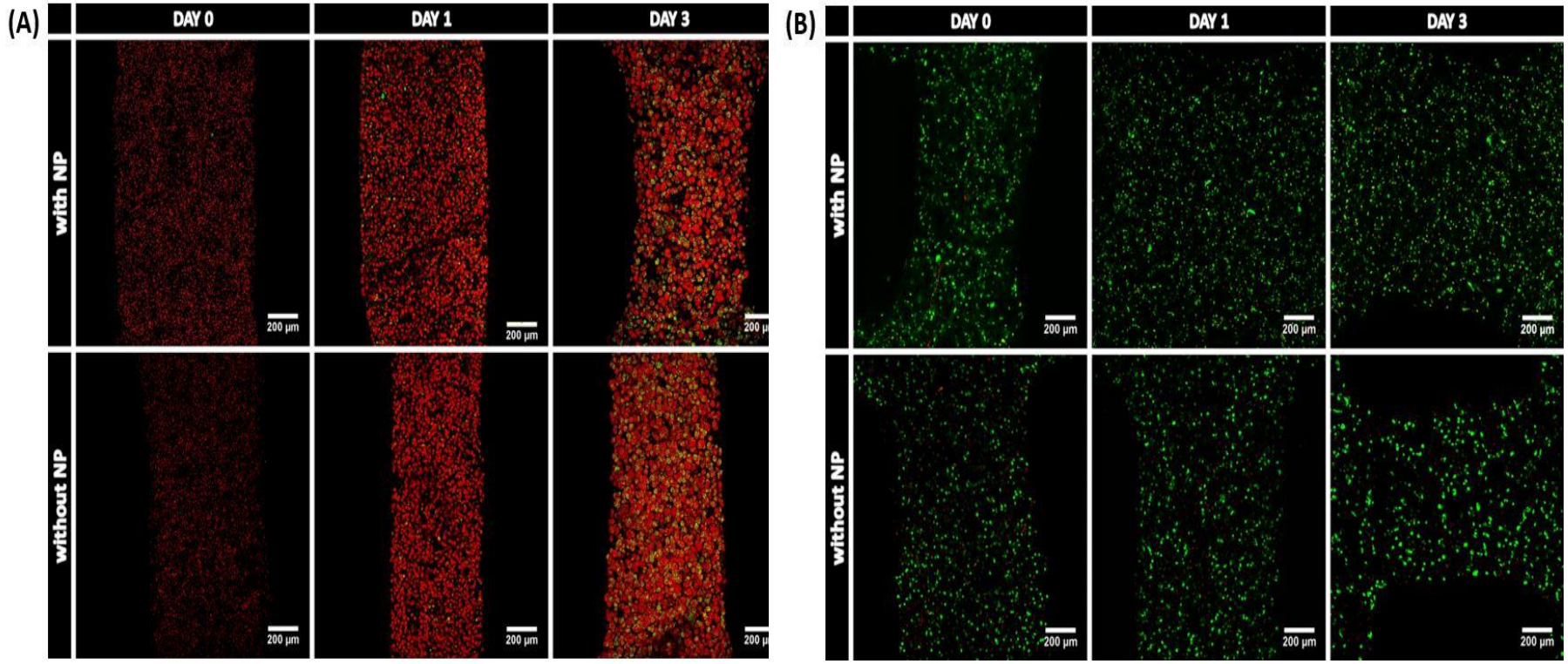


Figure 2. Viability of the microalga *Chlorella sorokiniana* and the mammalian cell line hTERT-MSC as a function of incubation time when immobilized in 3D-bioprinted hydrogel scaffolds containing O_2 sensor nanoparticles.



Viability of the microalga *Chlorella sorokiniana* (A) and the mammalian cell line (hTERTMSC) (B) when immobilized in 3D bioprinted alginate/methylcellulose scaffolds containing O_2 sensor nanoparticles. Assessment of viability was done by live-dead staining and fluorescence microscopy of 3D-printed cell-laden scaffolds after different incubations times.

Calibration of 3D-Printed Constructs with O_2-Sensitive Nanoparticles

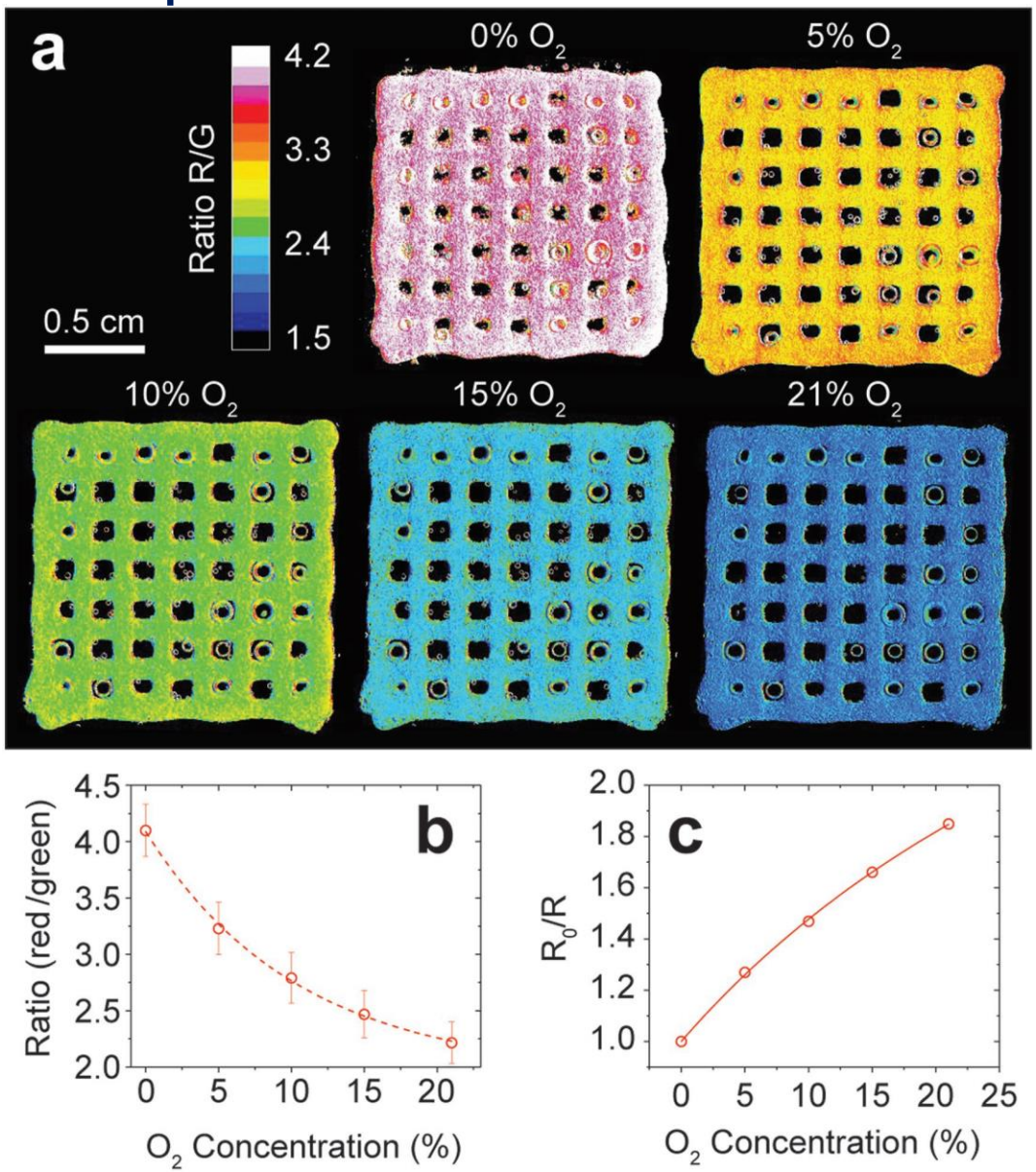


Figure 3. Calibration of a 3D-printed construct composed of hydrogel containing optical O_2 sensor nanoparticles. a) Ratio images (red channel/green channel) of a printed scaffold with nanoparticles incubated at different O_2 concentrations. b) Calibration curve obtained from the images in panel (a); values represent the mean of the entire scaffold with standard deviation, and dashed line shows a curve ft of a monoexponential decay function ($r2 > 0.998$). c) Stern–Volmer plot of the calibration curve ftted to Equation (1) ($r2 > 0.999$)

Effects of Cell Autofluorescence on O_2 Imaging

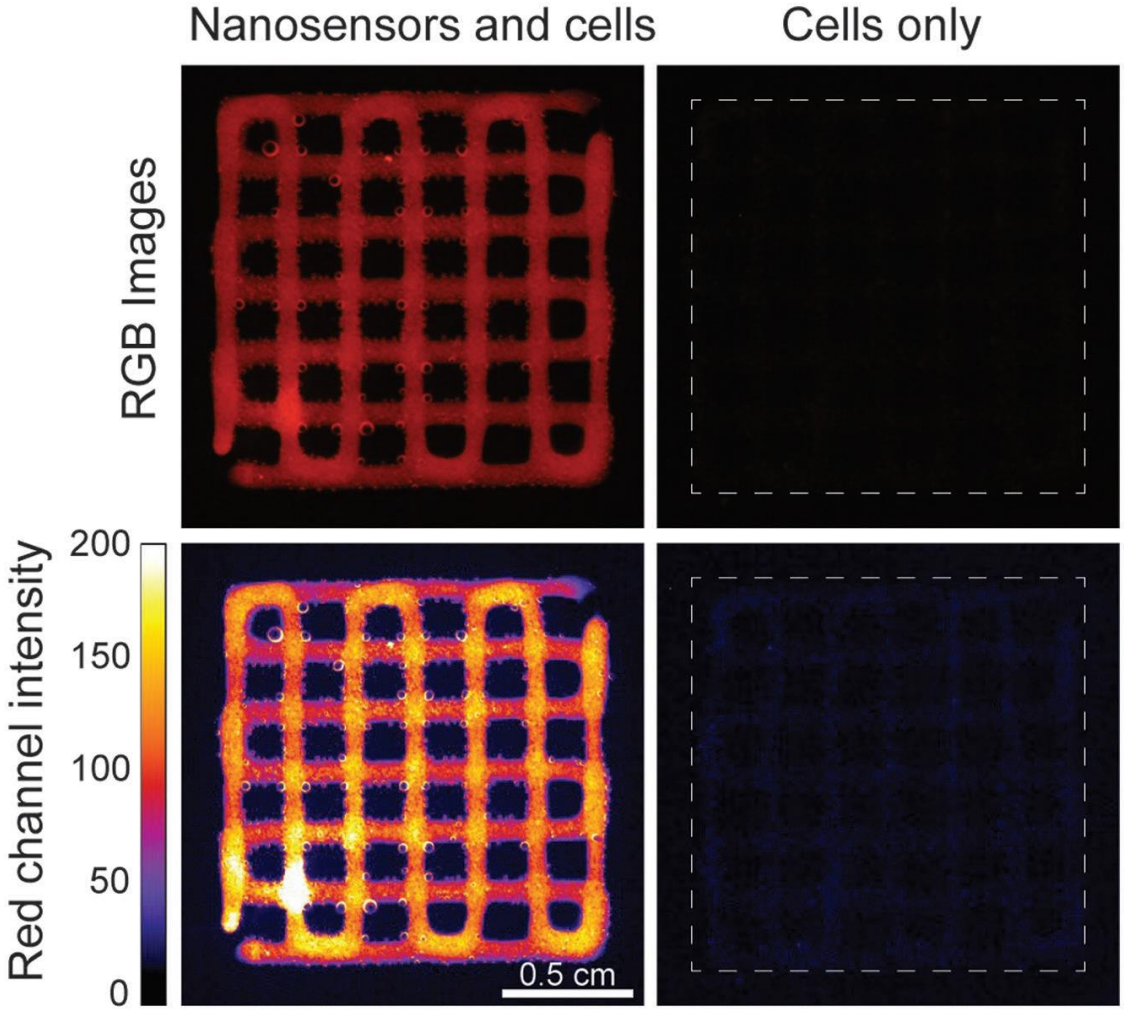
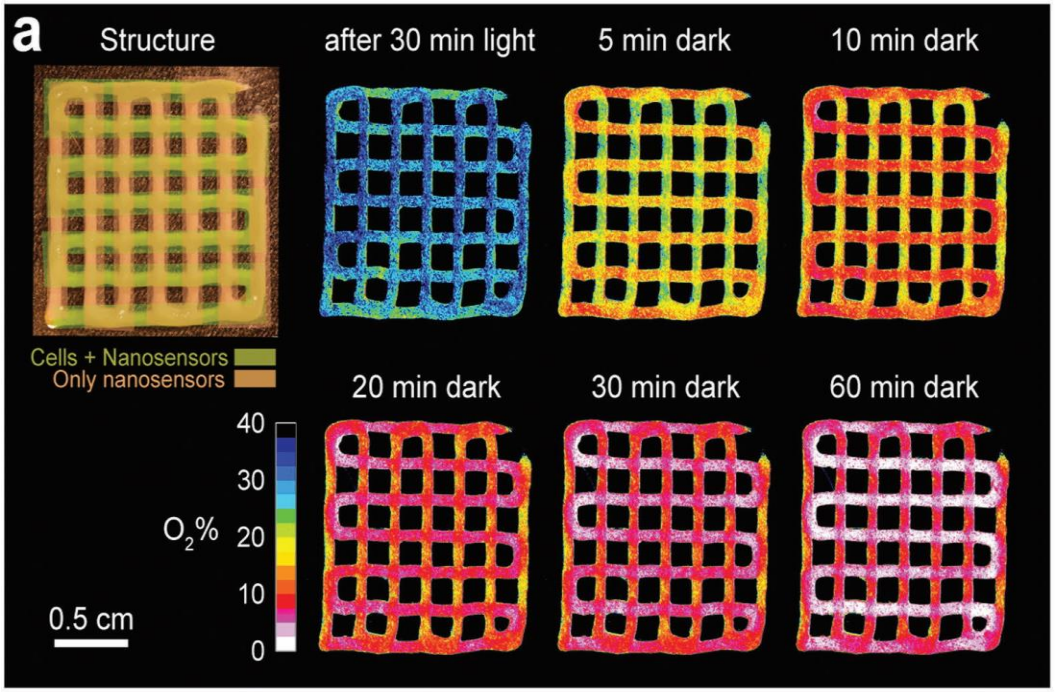


Figure 4. Background fluorescence of microalgae (*Chlorella sorokiniana*) as compared to the O_2-dependent luminescence signal from nanoparticles in the red channel of the recorded RGB image (false color bar). The bioprinted construct was incubated under a photon irradiance of 450 µmol photons m^{-2} s^{-1}.

Mapping Chemical Heterogeneity in 3D-Bioprinted Constructs



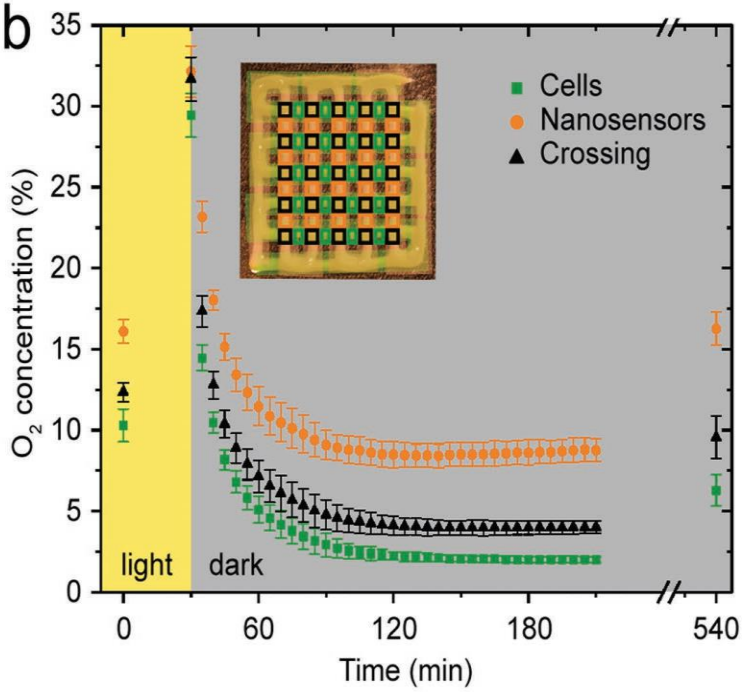


Figure 5. Spatiotemporal dynamics of O_2 concentration in a 3D-bioprinted construct consisting of one hydrogel layer of microalgae plus sensor nanoparticles (greenish horizontal layer) and a hydrogel layer of sensor nanoparticles only (vertical orange layer). a) Structural image of the hydrogel construct visualizing the two different layers and images of O_2 concentrations in the scaffold after 30 min illumination (450 µmol photons m^{-2} s^{-1}) and as a function of time after darkening. b) Time course of O_2 concentrations after a light-dark shift in different regions of interest as depicted in the inset (microalgae + nanoparticle layer, nanoparticle layer, crossings of the two layers).

Constructs with Microalgae, Mammalian Cells, and Sensor Nanoparticles

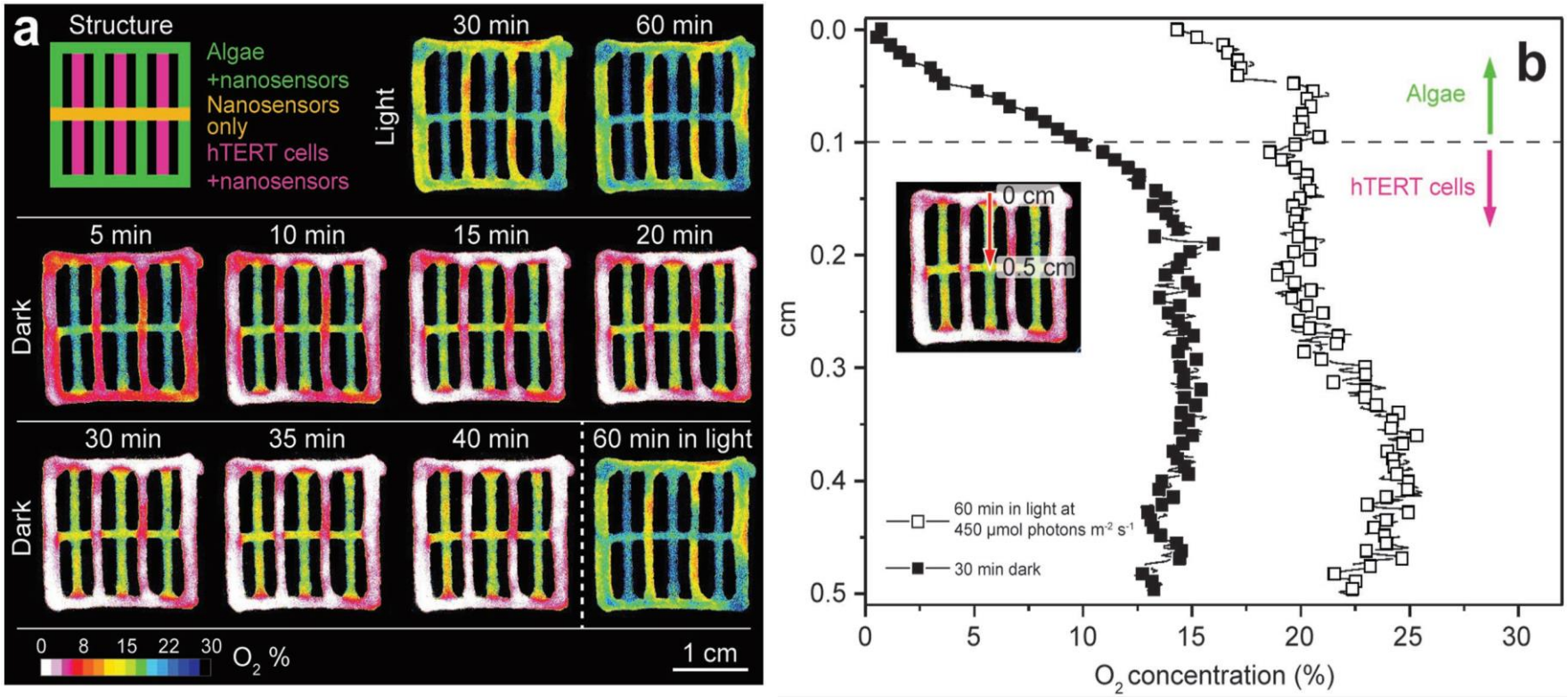


Figure 6. Spatiotemporal dynamics of O_2 concentration in a multilayered 3D bioprinted construct a) Visualization of the different scaffold layers and images of O_2 concentrations in the scaffold after 30 and 60 min exposure to a photon irradiance of 450 µmol photons m^{-2} s^{-1}, and as a function of time after subsequent darkening. b) Lateral profiles of O_2 concentration between hydrogel layer with microalgae + nanoparticles and a layer of hTERT-MSC + sensor nanoparticles measured after 60 min light exposure and 30 min of darkness, respectively.

Spatiotemporal Dynamics of O_2 Concentration in a Multilayered 3D-Bioprinted Construct

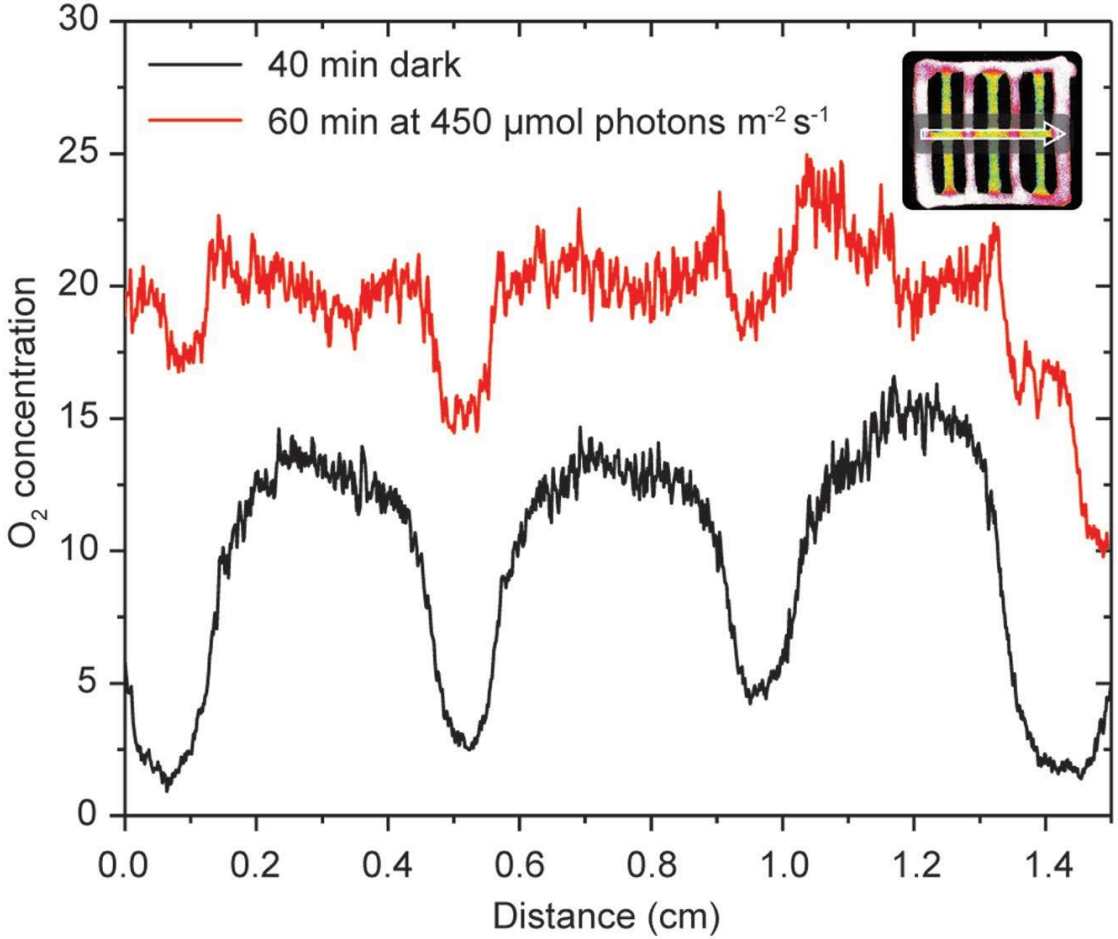


Figure 7. The graph shows extracted O_2 concentration profiles across the printed scaffold in a hydrogel strand containing only sensor nanoparticles measured after 60 min light exposure and 40 min of darkness, respectively.

Summary and Conclusions

- Developed a simple method to functionalize an alginate-based bioink with luminescent O2-sensing nanoparticles, which showed excellent printability and good biocompatibility when microalgae and/or mammalian cell-laden scaffolds were fabricated.

- Based on simple ratiometric luminescence imaging, demonstrated that the spatiotemporal dynamics of O2 concentration can be mapped across complex 3D-bioprinted constructs containing living cells.

- Use of such sensor-functionalized bioinks has a wide range of applications in 3D bioprinting and additive manufacturing, as it enables simple, rapid, and non-invasive mapping of the chemical microenvironment and activity of embedded cells in printed scaffolds; both as a function of external environmental factors such as light, temperature, salinity, pH, or nutrient/substrate availability, as well as due to cell–cell interactions in printed monocultures or in scaffolds with a more complex mixture of different cell types.

Thank you