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## Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering

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## Supplementary info

Part 1. Nucleotide sequences produced during cloning:

atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC CGGCGGCTTCGGCGCCAACGTCACGATCACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC CTACACCG

CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC TGCCCTGG

AACGGCAGCATCCCGACCGGCGGCACGGCGTCGTTCGGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG CCGGCGTC

GTTCTCGCTCAACGGCACCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtaccccAGCTT

**CBD** $\Delta$ (**FTN**) in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

atgagaggategcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC CGGCGGCTTCGGCGCCAACGTCACGATCACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC CTACACCG CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC TGCCCTGG AACGGCAGCATCCCGACCGGCGCACGGCGTCGTTCGGGTTCAACGGCTCGTGGGCCCGGGTCCAACCCGACG CCGGCGTC GTTCTCGCTCAACGGCACCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtaccGGTACAGGTGGTT CTGGTG GTGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACG GCCACAAG TTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACC GGCAAGCT GCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCA CATGAAGC AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGG CAACTAC AAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTC AAGGAGGA CGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACATCAGCCACAACGTCTATATCACCGCCGACAAGCAG AAGAACG GCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCA GAACACC CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGAC CCCAACGA GAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTAC AAGTCCG GACTCAGATCTCGAGCTCAgGCTTCGAATTCTGCAGTCGACGGcACCGCGGGCccAggaTCtACCGGATCTAGAggt acc cccAGCTT

**CBD**(**G**)-**ECFP** in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWTYTAGQRIQQLWNGTASTNGGQVS VTSLPW

NGSIPTGGTASFGFNGSWAGSNPTPASFSLNGTTCTGTVPTTSPTGT<mark>GTGGSGG</mark>VSKGEELFTGVVPILVELDGDVN GHK

 ${\sf FSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD}{\sf GNY}$ 

KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYISHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNT PIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKSGLRSRAQASNSAVDGTAGPGSTGS RGT PS

Primary structure of **CBD**(**G**)-**ECFP protein**. 402 aa, 43,1 kDa, pI 6.6. Gly-rich linker is highlighted in yellow.

atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC CGGCGGCTTCGGCGCCAACGTCACGATCACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC CTACACCG CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC TGCCCTGG AACGGCAGCATCCCGACCGGCGCACGGCGTCGTTCGGGTTCAACGGCTCGTGGGCCCGGGTCCAACCCGACG CCGGCGTC GTTCTCGCTCAACGGCACCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACG<mark>ggtacc</mark>GGTACAGGTGGTT CTGGTG GTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCTGTACGACGATGACGATAAGGATCTCGCCAC CATGGTC GACTCATCACGTCGTAAGTGGAATAAGACAGGTCACGCAGTCAGAGCTATAGGTCGGCTGAGCTCACTCGAGAA CGTCTA TATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAGGACGGCGGC GTGCAGC TCGCCTACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCCCGACAACCACTACCTGAG CACCCAG TCCAAACTTTCGAAAGACCCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGA TCACTCT CGGCATGGACGAGCTGTACAAGGGCGGcACCGGAGGAGCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGG GGTGGTGC CCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGTGAGGGCGATG CCACCTAC GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCC TGACCTA CGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAA GGCTACA TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGA CACCCTG GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA ACACGCG TGACCAACTGACTGAAGAGCAGATCGCAGAATTTAAAGAGGCTTTCTCCCTATTTGACAAGGACGGGGATGGGA CAATAA CAACCAAGGAGCTGGGGACGGTGATGCGGTCTCTGGGGCAGAACCCCACAGAAGCAGAGCTGCAGGACATGA TCAATGAA GTAGATGCCGACGGTAATGGCACAATCGACTTCCCTGAGTTCCTGACAATGATGGCAAGAAAAATGAAAGACAC AGACAG TGAAGAAGAAATTAGAGAAGCGTTCCGTGTGTTTGATAAGGATGGCAATGGCTACATCAGTGCAGCAGAGCTTC GCCACG TGATGACAAACCTTGGAGAGAAGTTAACAGATGAAGAGGTTGATGAAATGATCAGGGAAGCAGACATCGATGGG GATGGT CAGGTAAACTACGAAGAGTTTGTACAAATGATGACAGCGAAG CBD(G)-GCaMP2 in pQE-30 vector (indicated in grey). Kpn I site used for cloning fusions and linker peptide is indicated in vellow. MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWTYTAGQRIQQLWNGTASTNGGQVS VTSLPW NGSIPTGGTASFGFNGSWAGSNPTPASFSLNGTTCTGTVPTTSPTGTGTGTGGGGGMASMTGGQQMGRDLYDDDDK DLATMV DSSRRKWNKTGHAVRAIGRLSSLENVYIMADKQKNGIKANFKIRHNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLS TQ SKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGTGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGD ATY GKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGD

GKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGD TL VNRIELKGIDFKEDGNILGHKLEYNTRDQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMIN

E VDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDG QVNYEEFVQMMTAKGTPS Primary structure of **CBD**(**G**)-**GCaMP2** protein. 578 aa, 63,2 kDa, pI 4.9. Gly-rich linker is highlighted in yellow.

**Table S1.** Primer pairs used for cloning of ECFP and GCaMP2.

| ECFP (no linker peptide)   | Forward: 5'-GATCGGTACCGTGAGCAAGGGCGAGGA           |
|----------------------------|---|
|                            | Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT         |
| GCaMP2 (no linker peptide) | Forward: 5'-GATCGGTACCATGGCTAGCATGACTGGTGGAC      |
|                            | Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTTGTA        |
|                            |   |
| ECFP (with linker peptide  | Forward: 5'-                                      |
| GTGGSGG)                   | GATCGGTACCGGTACAGGTGGTGGTGGTGGTGGTGAGCAAGGGCGAGGA |
|                            | Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT         |
| GCaMP2 (with linker        | Forward: 5'-                                      |
| peptide GTGGSGG)           | AGTCGGTACCGGTACAGGTGGTTCTGGTGGTATGGCTAGCATGACTGGT |
|                            | Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTTGTA        |
|                            |   |

Part 2. Supplementary figures



**Figure S1.** Fluorescence lifetime decays for ECFP and CBD-ECFP proteins measured in solution at different pH. A: Observed decay times for ECFP were 1.35 ns (pH 4.5) and 2 ns (pH 8). B: Observed decay times for CBD-ECFP were 1.89 ns (pH 4.5) and 2.18 ns (pH 8). Standard deviations are shown in gray. Prompt (background) fluorescence signals are also shown. N=3.



**Figure S2.** Comparison of specific / non-specific staining of GrowDex with CBD-ECFP (A) and CBD-GCaMP2 proteins (B) compared to untagged ECFP and GCaMP2 proteins. Matrices were incubated with 10  $\mu$ M of respective proteins for 15 min in PBS, washed and measured on fluorescence microscope (470 nm exc., 510-560 nm em.). N=3.



**Figure S3.** A: Concentration-dependent staining of GrowDex with CBD-GCaMP2 (incubation time 15 min, in PBS). B: Stability of stained (5  $\mu$ M, 15 min) GrowDex scaffold. Matrices were measured on a fluorescence microscope (470 nm exc., 510-560 nm em.), using the same acquisition settings for different experimental points. N=3.



**Figure S4.** Characterization of bacterial cellulose. A: Photograph of cellulose sphere produced by *Gluconacetobacter hansenii* GH-1/2008 (VKPM B-10547). B: 3D microscopy and nanoindentation reveals homogenous surface of the bacterial cellulose. Young's modulus is 1.93±0.76 kPa.



**Figure S5.** Overview of plant decellularization (DC) procedure and studied examples. A: cartoon representation of the use of DC plant for cell-based application with CBD-tagged biosensors. B: Photographs of celery stem section before and after decellularization. 1 euro coin is shown for scale. C: Transmission light and widefield fluorescence (470 nm exc., 510-560 nm em.) microscopy images for spinach leaf and celery stem before (intact), after DC and following labeling either with ECFP (non-specific binding) or CBD-ECFP. The same acquisition settings were used for fluorescence images. Scale bar is in µm.



**Figure S6.** Staining and stability of decellularized (DC) celery and spinach. DC samples were stained with various concentrations of CBD-ECFP or ECFP and measured on a fluorescence microscope (470 nm exc., 510-560 nm em.). A: Concentration-dependence of staining of DC spinach (16 h incubation in PBS). B: Storage stability of stained DC spinach (5  $\mu$ M, 16 h) over 2 weeks time. C: Staining of DC celery with CBD-ECFP (5  $\mu$ M, 16 h). N=3.



**Figure S7.** Cell growth in unstained and CBD-ECFP-stained Growdex. HCT116 cells were seeded in stained (5  $\mu$ M CBD-ECFP, 15 min) or unstained (control) Growdex matrices, grown for 0-7 days and quantified by using fluorescence microscopy with the help of Hoechst 33342 (1  $\mu$ M, 30 min) staining. A: Fluorescence microscopy images showing cell growth (CBD-ECFP-stained sample). TL-transmission light. Scale bar is 50  $\mu$ m. B: Calculated cell

numbers (per region of interest) for different time points of culture in Growdex. No significant differences (indicated as 'NS') between CBD-ECFP-stained and unstained samples were found for mean values (*t*-test, p=0.05). Cells grown in Growdex cells also formed spheroids over time (shown as green and red dashed lines, red Y axis). N=3.



**Figure S8.** Dependence of extracellular acidification rates from cell number, measured on a microplate reader by ECA method. HCT116 cells seeded and grown at different densities (10,000, 50,000 and 100,000 designated as 10K, 50K and 100K, respectively) were measured by ECA method (unsealed system, glycolytic flux) in the presence of 1  $\mu$ M FCCP and 10  $\mu$ M Oligomycin (FCCP-OM, maximal acidification) and DMSO (mock). A: time-dependence of extracellular acidification. B: extracellular acidification rates (top) and average total protein concentrations (bottom). N=8. Asterisks indicate significant difference (*t*-test, p=0.001)