

Title	Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering
Authors	O'Donnell, Neil;Okkelman, Irina A.;Timashev, Peter;Gromovykh, Tatyana I.;Papkovsky, Dmitri B.;Dmitriev, Ruslan I.
Publication date	2018-09-25
Original Citation	O'Donnell, N., Okkelman, I. A., Timashev, P., Gromovykh, T. I., Papkovsky, D. B. and Dmitriev, R. I. (2018) 'Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering', Acta Biomaterialia, 80, pp. 85-96. doi:10.1016/j.actbio.2018.09.034
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.actbio.2018.09.034
Rights	© 2018, Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - https://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2024-12-14 01:42:50
Item downloaded from	https://hdl.handle.net/10468/7080



UCC

University College Cork, Ireland
 Coláiste na hOllscoile Corcaigh

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

Neil O'Donnell^{1*}, Irina A. Okkelman^{1*}, Peter Timashev^{2,3}, Tatyana I. Gromovykh⁴, Dmitri B. Papkovsky¹, Ruslan I. Dmitriev^{1,2}

¹ School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland.

² Institute for Regenerative Medicine, I.M. Sechenov First Moscow State University, Moscow, Russian Federation

³ Institute of Photonic Technologies, Research Center 'Crystallography and Photonics, Russian Academy of Sciences, Moscow, Russian Federation

⁴ Department of Biotechnology, I.M. Sechenov First Moscow State University, Moscow, Russian Federation

Supplementary info

Part 1. Nucleotide sequences produced during cloning:

```
atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCCGCTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCTGTTCCGGTTCAACGGCTCGTGGGCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGTCAACGGCACCCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtacccccAGCTT
```

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

```
atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATACCAACCTCGGCGACCCCGTCTCGTCGTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCCGCTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCTGTTCCGGTTCAACGGCTCGTGGGCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGTCAACGGCACCCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtaccGGTACAGGTGGTT
CTGGTG
GTGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACG
GCCACAAG
TTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACC
GGCAAGCT
GCCCCGTGCCCTGGCCACCCTCGTGACCACCCTGACCTGGGGCGTGACGTGCTTACGCCGCTACCCCGACCA
CATGAAGC
AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGG
CAACTAC
AAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTC
AAGGAGGA
CGGCAACATCCTGGGGACAAGCTGGAGTACAACCTACATCAGCCACAACGTCTATATCACCGCCGACAAGCAG
AGAACG
GCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCA
GAACACC
CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGAC
CCCAACGA
GAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
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.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWYTAGQRIQLWNGTASTNNGQVS
VTSLPW
NGSIPTGGTASFGFNWSWAGSNPTPASFSLNGTTCTGTVPPTSPTGTGTGGSGG VSKGEELFTGVVPILVELDGDVN
GHK
FSVSGEGEGDATYGLTLKFICTTGKLPVPWPTLVTTLTWGVQCFSTRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD
GNY
KTRAEVKFEGDNLVNRIELKIDFKEDGNILGHKLEYNRYISHNVYITADKQKNGIKANFKIRHNIEDGVSQVLADHYQQNT
PIGDGPVLLPDNHYLSTQSALS KDPNEKRDMVLEFVTAAGITLGMDELYKSGLSRAQASNSAVDGTAGPGSTGS
RGT
PS

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

atgagaggatcgcaccatcaccatcaccggtaccGCTCCCGGCTGCCGCTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATACCAACCTCGGCGACCCCGTCTCGTCTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCGCGTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCTCGGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGTCAACGGCACCCACTGCACGGGCACCGTCCGACGACCAGCCCCACGggtaccGGTACAGGTGGTT
CTGGTG
GTATGGCTAGCATGACTGGTGGACAGAAATGGGTCCGGGATCTGTACGACGATGACGATAAGGATCTCGCCAC
CATGGTC
GACTCATACGTCGTAAGTGAATAAGACAGGTCACGCAGTCAGAGCTATAGGTCCGGCTGAGCTCACTCGAGAA
CGTCTA
TATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAGGACGGCGGC
GTGCAGC
TCGCCTACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAG
CACCCAG
TCCAACTTTTCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGA
TCACTCT
CGGCATGGACGAGCTGTACAAGGGCGGcACCGGAGGGAGCATGGTGAAGGCGAGGAGCTGTTACCCGG
GGTGGTGC
CCATCCTGGTCGAGCTGGACGGCGACGTAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGTGAGGGCGATG
CCACCTAC
GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGCAAGCTGCCCGTGCCCTGGCCACCCTCGTGACCACCC
TGACCTA
CGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCGAA
GGCTACA
TCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGA
CACCCTG
GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA
ACACGCG
TGACCAACTGACTGAAGAGCAGATCGCAGAATTTAAAGAGGCTTTCTCCCTATTTGACAAGGACGGGGATGGGA
CAATAA
CAACCAAGGAGCTGGGGACGGTGTGCGGTCTCTGGGGCAGAACCCACAGAAGCAGAGCTGCAGGACATGA
TCAATGAA
GTAGATGCCGACGGTAATGGCACAATCGACTTCCCTGAGTTCCTGACAATGATGGCAAGAAAAATGAAAGACAC
AGACAG
TGAAGAAGAAATTAGAGAAGCGTTCCTGTGTTTGATAAGGATGGCAATGGCTACATCAGTGCAGCAGAGCTTC
GCCACG
TGATGACAAACCTTGGAGAGAAGTTAACAGATGAAGAGGTTGATGAAATGATCAGGGAAGCAGACATCGATGGG
GATGGT
CAGGTAAACTACGAAGAGTTTGTACAAATGATGACAGCGAAGggtacccccAGCTT

CBD(G)-GCaMP2 in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWYTAGQRIQLWNGTASTNNGQVS
VTSLPW
NGSIPTGGTASFGFNWSWAGSNPTPASFSLNGTTCTGTVPPTSPTGTGTGGSGG MASMTGGQQMGRDLYDDDDK
DLATMV
DSSRRKWNKTGHAVRAIGRLSSENVYIMADKQKNGIKANFKIRHNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLS
TQ
SKLSKDPNEKRDMVLEFVTAAGITLGMDELYKGGTGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGD
ATY
GKLT LKFICTTGKLPVPWPTLVTTLTWGVQCFSTRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGD
TL
VNRIELKIDFKEDGNILGHKLEYNTRDQLTEEQIAEFKEAFSLFDKDGDTITTKELGTVMRSLGQNPTAEALQDMIN
E
VDADGNGTIDFPEFLTMMARKMKD TDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDG
DG

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'-GATCGGTACCCTTCGCTGTCATCATTGTA
ECFP (with linker peptide GTGGS GG)	Forward: 5'- GATCGGTACCGGTACAGGTGGTTCTGGTGGTGTGAGCAAGGGCGAGGA Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT
GCaMP2 (with linker peptide GTGGS GG)	Forward: 5'- AGTCGGTACCGGTACAGGTGGTTCTGGTGGTATGGCTAGCATGACTGGT Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTGTA

Part 2. Supplementary figures

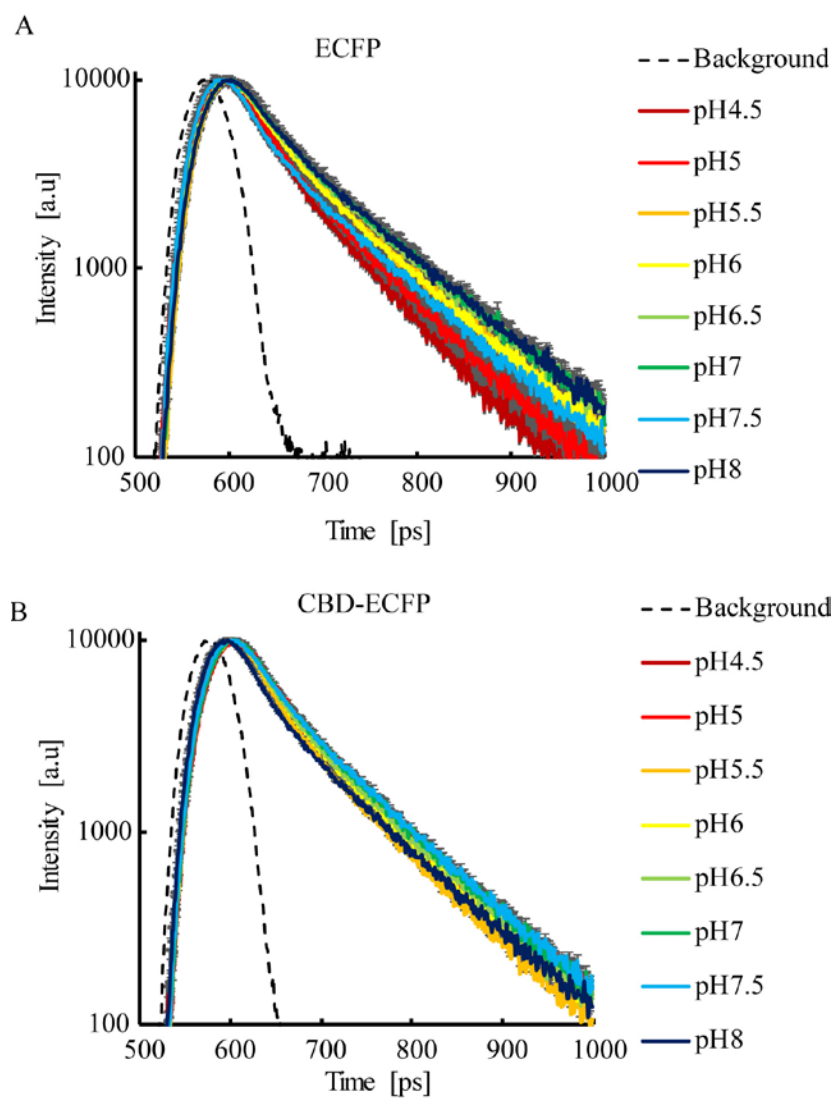


Figure S1. Fluorescence lifetime decays for ECFP and CBD-ECFP proteins measured in solution at different pH. A: Observed decay times for ECFP were 1.35 ns (pH 4.5) and 2 ns (pH 8). B: Observed decay times for CBD-ECFP were 1.89 ns (pH 4.5) and 2.18 ns (pH 8). Standard deviations are shown in gray. Prompt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

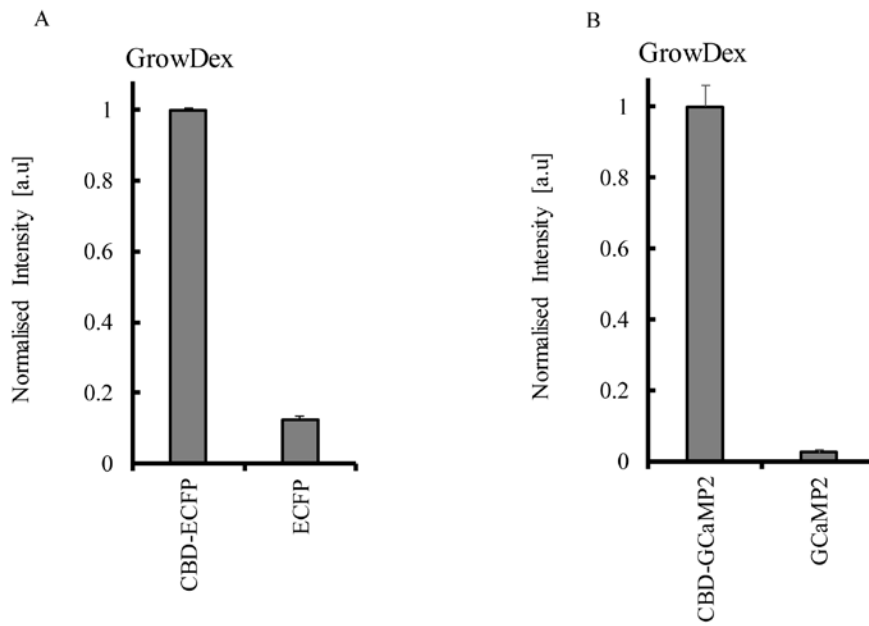


Figure S2. Comparison of specific / non-specific staining of GrowDex with CBD-ECFP (A) and CBD-GCaMP2 proteins (B) compared to untagged ECFP and GCaMP2 proteins. Matrices were incubated with 10 μM of respective proteins for 15 min in PBS, washed and measured on fluorescence microscope (470 nm exc., 510-560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

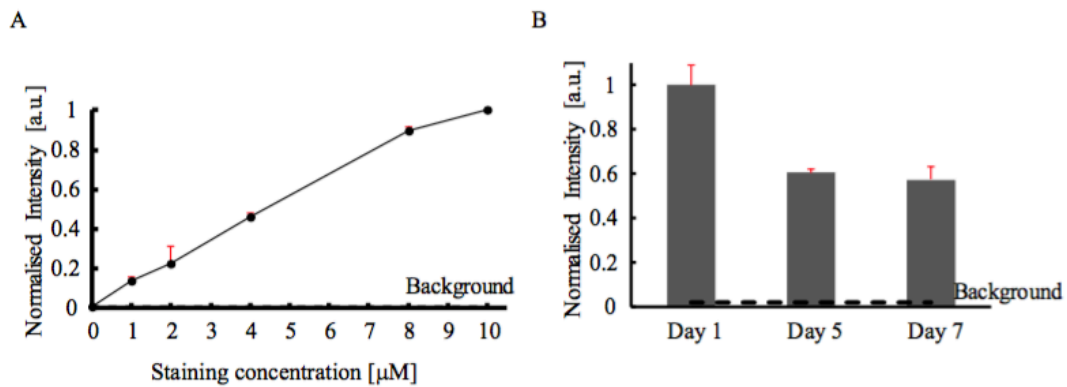


Figure S3. A: Concentration-dependent staining of GrowDex with CBD-GCaMP2 (incubation time 15 min, in PBS). B: Stability of stained (5 μM , 15 min) GrowDex scaffold. Matrices were measured on a fluorescence microscope (470 nm exc., 510-560 nm em.), using the same acquisition settings for different experimental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

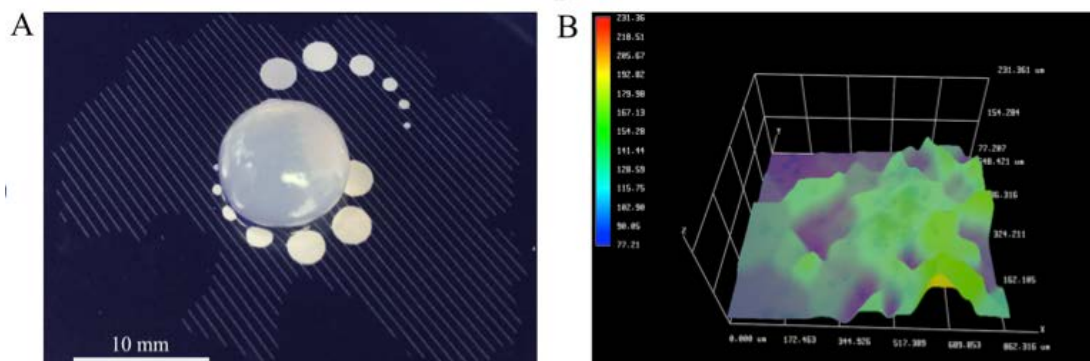


Figure S4. Characterization of bacterial cellulose. A: Photograph of cellulose sphere produced by *Gluconacetobacter hansenii* GH-1/2008 (VKPM B-10547). B: 3D microscopy and nanoindentation reveals homogenous surface of the bacterial cellulose. Young's modulus is 1.93 ± 0.76 kPa.

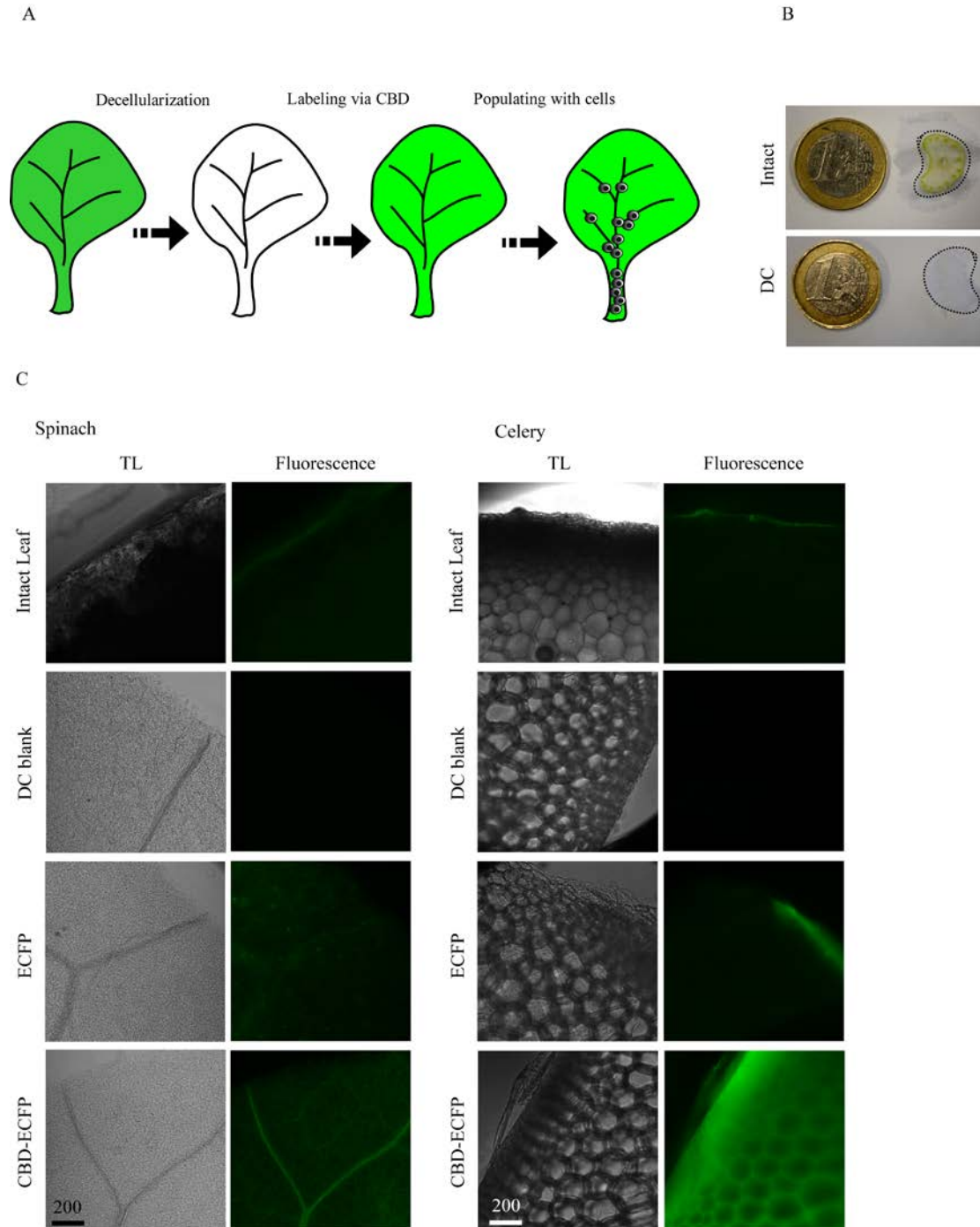


Figure S5. Overview of plant decellularization (DC) procedure and studied examples. A: cartoon representation of the use of DC plant for cell-based application with CBD-tagged biosensors. B: Photographs of celery stem section before and after decellularization. 1 euro coin is shown for scale. C: Transmission 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 .

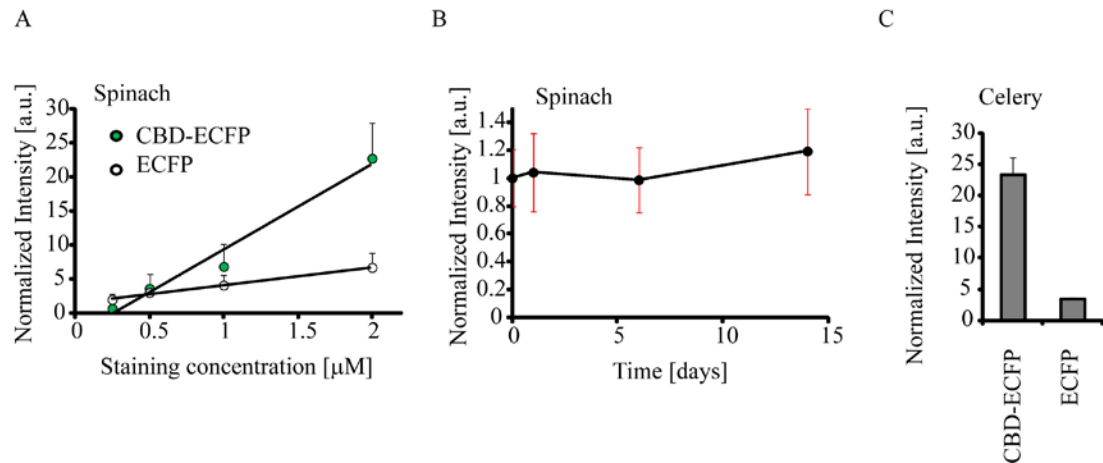


Figure S6. Staining and stability of decellularized (DC) celery and spinach. DC samples were stained with various concentrations of CBD-ECFP or ECFP and measured on a fluorescence microscope (470 nm exc., 510-560 nm em.). A: Concentration-dependence of staining of DC spinach (16 h incubation in PBS).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

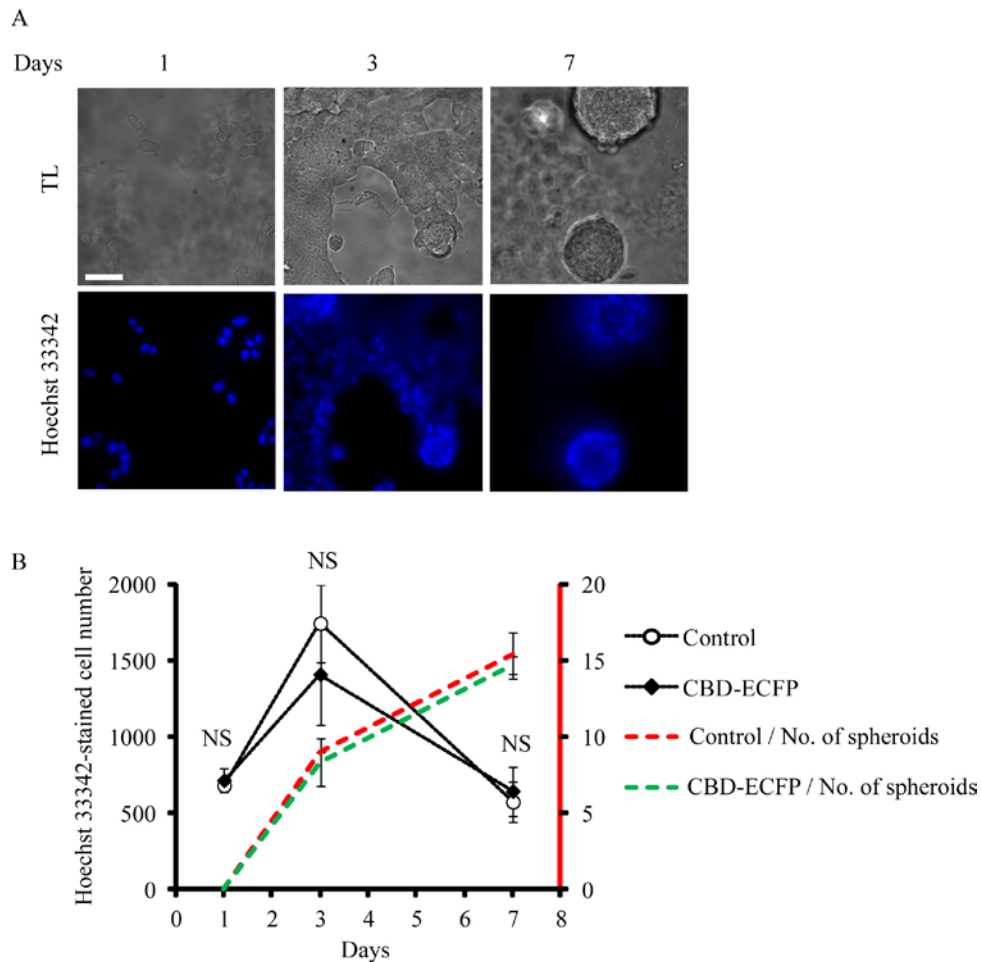


Figure S7. Cell growth in unstained and CBD-ECFP-stained Growdex. HCT116 cells were seeded in stained (5 μ M CBD-ECFP, 15 min) or unstained (control) Growdex matrices, grown for 0-7 days and quantified by using fluorescence microscopy with the help of Hoechst 33342 (1 μ M, 30 min) staining. A: Fluorescence microscopy images showing cell growth (CBD-ECFP-stained sample). TL-transmission light. Scale bar is 50 μ 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). $N=3$.

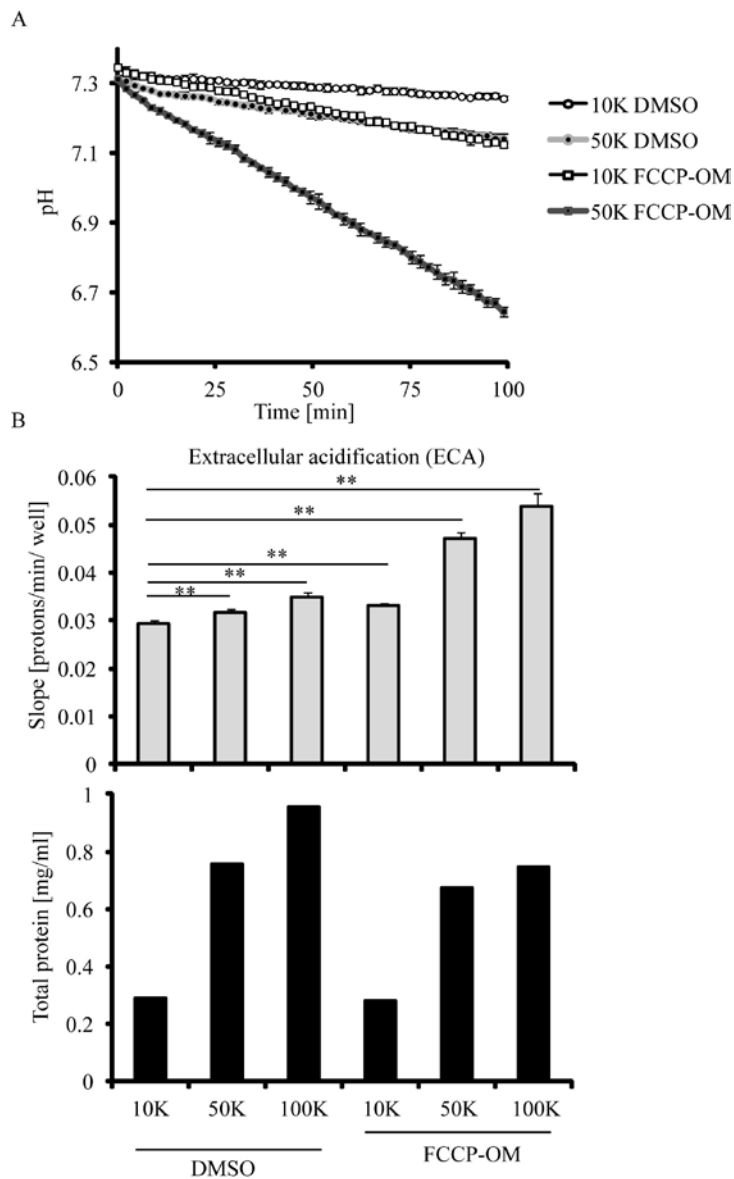


Figure S8. Dependence of extracellular acidification rates from cell number, measured on a microplate reader by ECA method. HCT116 cells seeded and grown at different densities (10,000, 50,000 and 100,000 designated as 10K, 50K and 100K, respectively) were measured by ECA method (unsealed system, glycolytic flux) in the presence of 1 μ M FCCP and 10 μ 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, $p=0.001$)