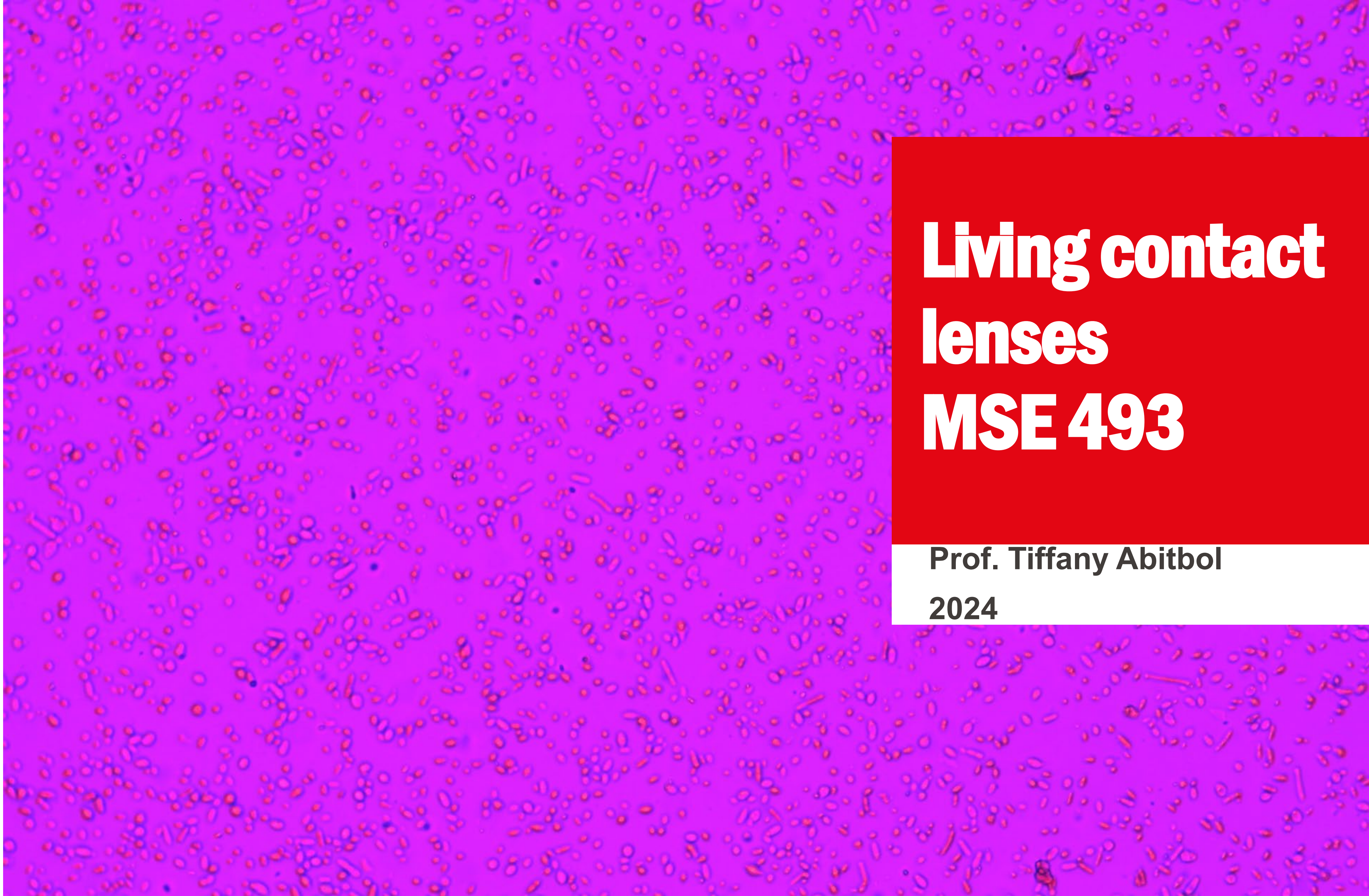
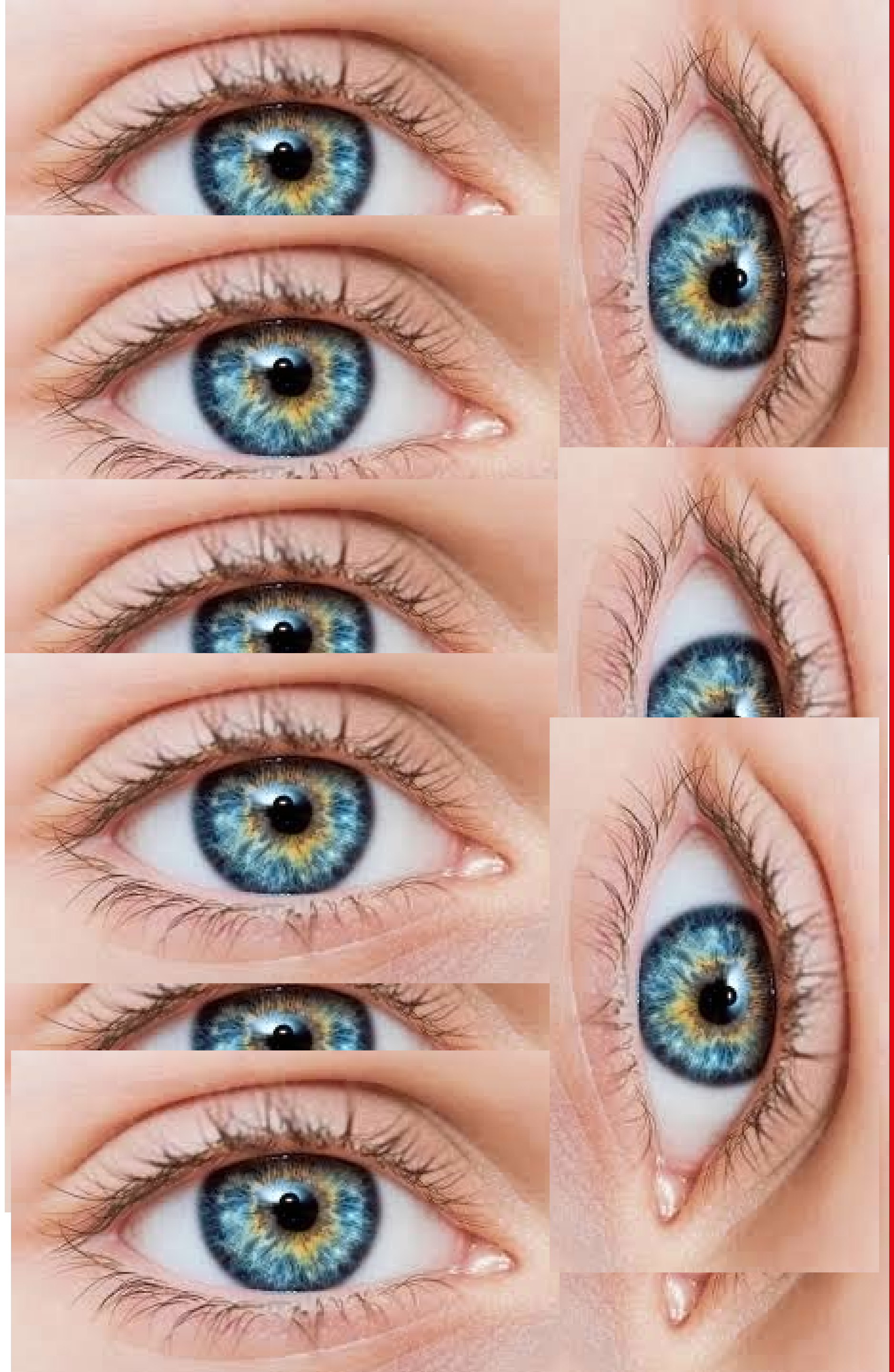


Living contact lenses MSE 493

Prof. Tiffany Abitbol

2024

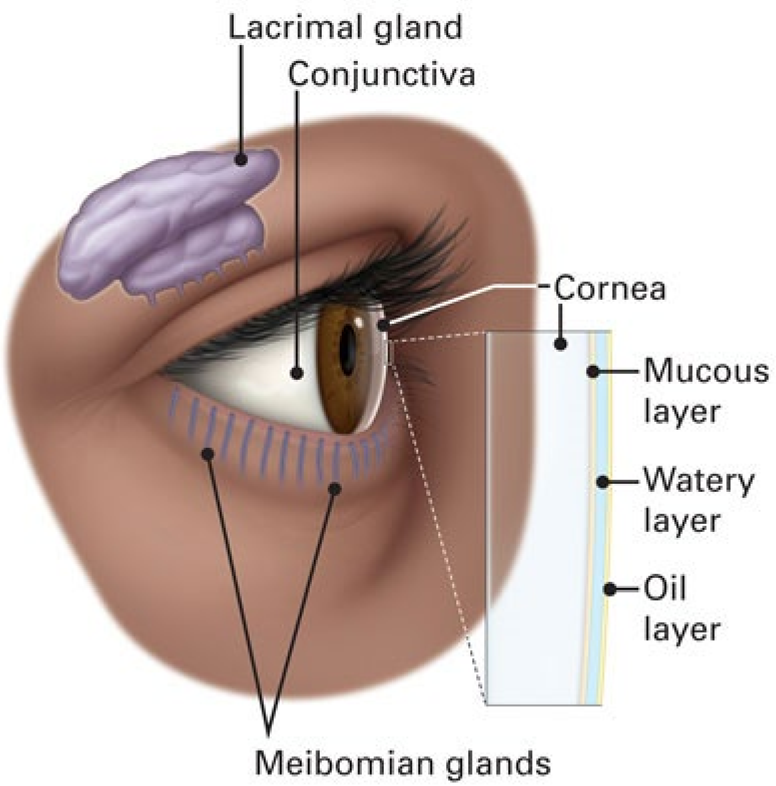
EPFL



Background

The eye, corrective lenses, dry eye

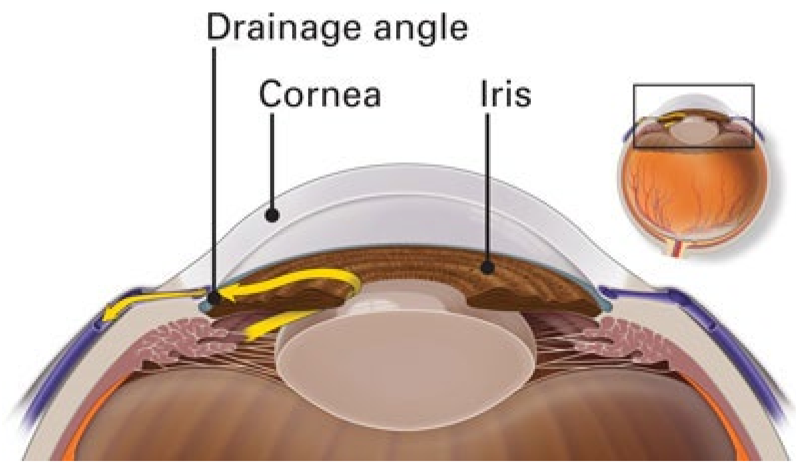
Anatomy of the eye



- Surface of eye and inner eyelid are covered by a clear membrane called a conjunctiva
- Tear film consists of 3 layers
  - Mucous layer (conjunctiva)
  - Watery layer – produced by lacrimal gland
  - Oil layer – produced by meibomian gland (keeps tears from drying out too quickly)
- Tears drain from eyes from the tear duct

[Anatomy of the eye]

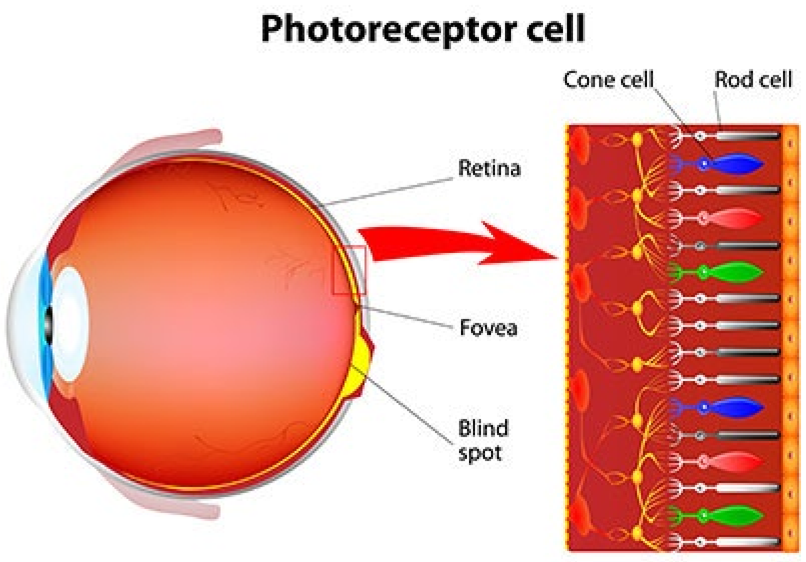
Anatomy of the eye – front of the eye



- Light is focused into the eye through the cornea (transparent, dome-shaped)
- Anterior chamber behind the eye filled with fluid called aqueous humor – constant eye pressure (balance of production and drainage)
- Iris and pupil behind anterior chamber – iris (colored part of eye) has muscles that dilate and constrict to open and close the pupil and control the light coming to the back of the eye
- The lens is right behind the pupil – the lens changes shape to help the focus the light entering the eye, toward the back of the eye

[Anatomy of the eye]

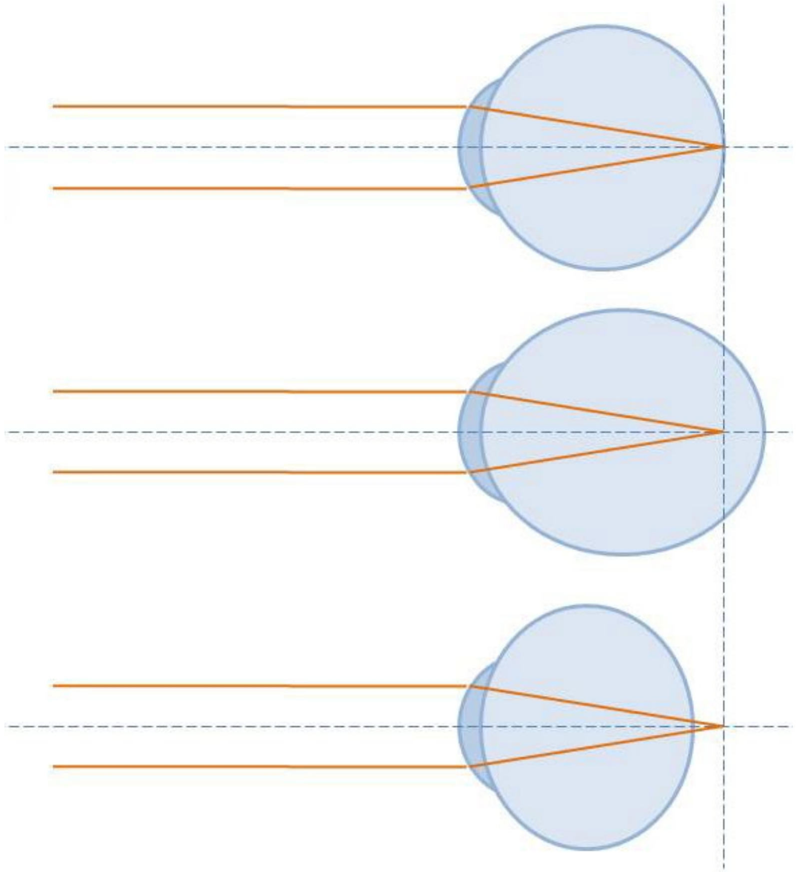
Anatomy of the eye – back of the eye



- Vitreous cavity between the lens and back of eye, filled with the vitreous humor (jelly-like)
- Light focused onto the retina by cornea and lens onto retina
- Retina is a light-sensitive tissue that lines the back of the eye
- Photoreceptors in the retina change lights into energy that is transmitted to the brain via the optical nerve
- Photoreceptors: Rods and cones
- Rods – black/white
- Cones – color

Anatomy of the eye

Eyesight



Correctly focused

Focused in front of retina
Near-sighted (light refracted too much)

Focal point behind retina
Far-sighted (light refracted too little)

[Refractive error]

OCTOBER 1, 2024 | 7 MIN READ

The Nearsightedness Epidemic Has Become a Global Health Issue

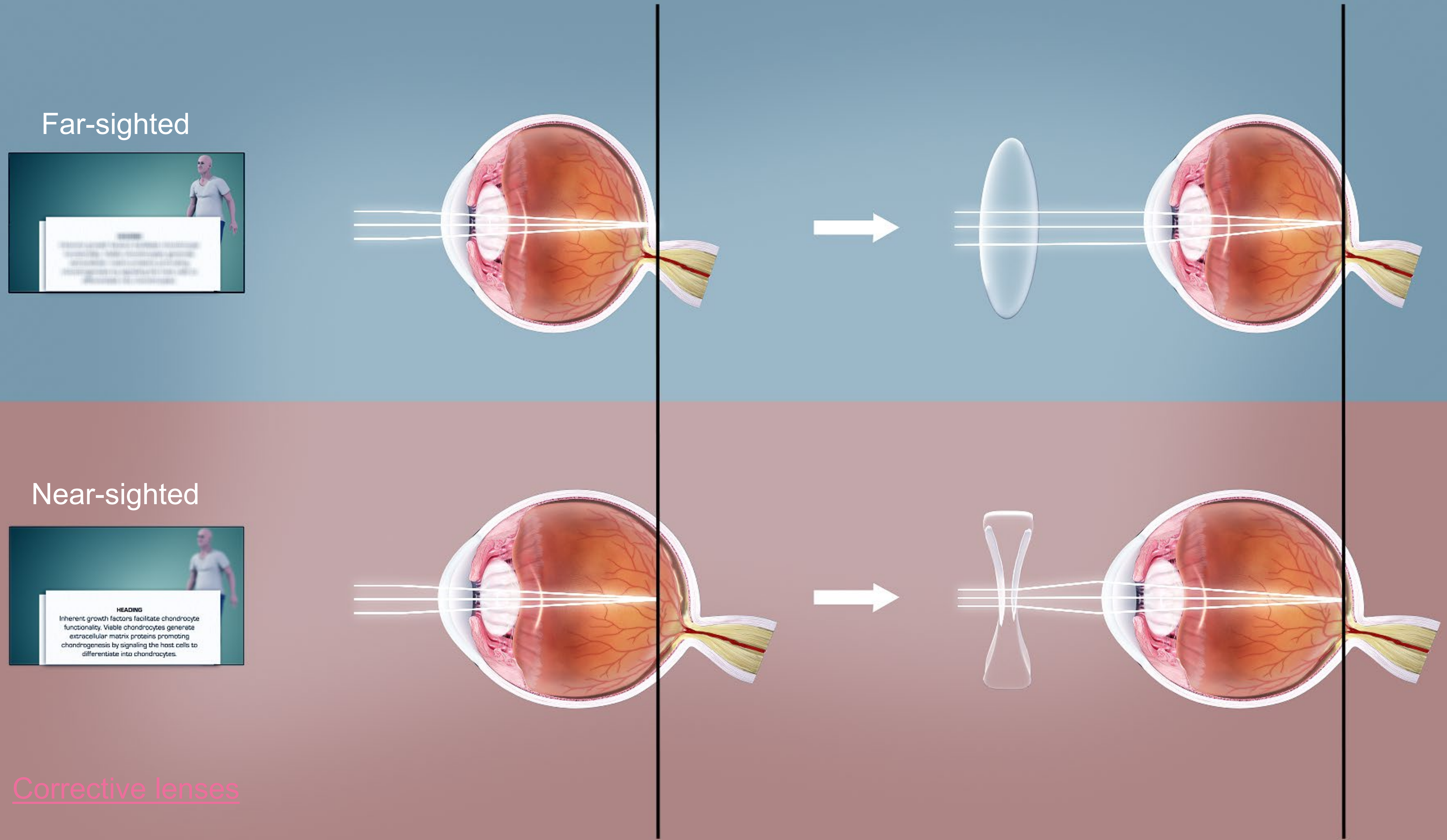
Myopia is projected to affect half of the world's population by 2050. A new report says it needs to be countered by classifying it as a disease and upping children's outdoor time

BY GARY STIX

Why? Too much time spent indoors
Outdoor light has a more varied "visual diet"

https://www.scientificamerican.com/article/nearsightedness-has-become-a-global-health-issue/

Corrective lenses



Corrective lenses

Dry eyes

Symptoms

- Not enough tears produced
- Tears evaporate too quickly

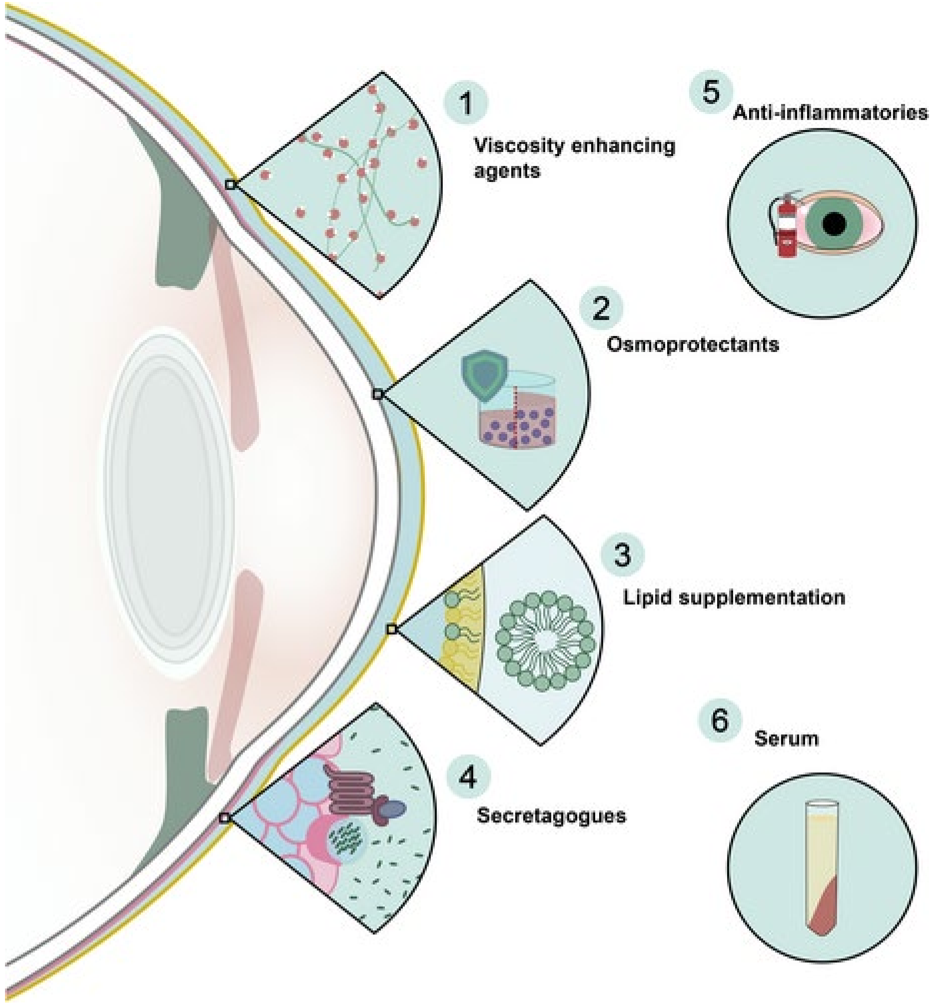
- Dryness, redness, discharge
- Blurred vision
- Tear film instability
- Increased risk to damage the ocular surface

Causes

- Age
- Various deficiencies/illnesses
- Side effects of medications
- Laser surgery
- Contact lenses
- Screen use (we blink less)

https://onlinelibrary.wiley.com/doi/full/10.1111/aos.15675

Dry eyes – topical treatments

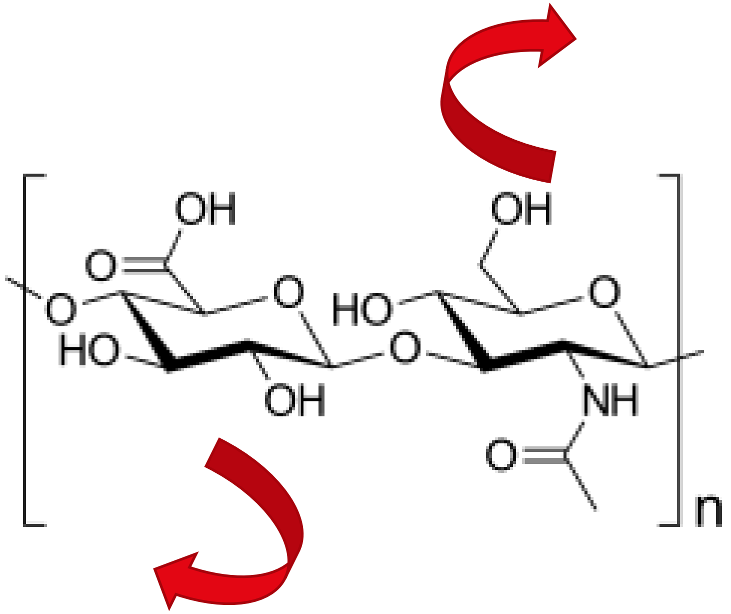


- Natural and synthetic polymers and emollients
- Benefits to ocular surface:
  - Improved viscosity
  - Improved surface adhesion
  - Improved tear-film distribution, lubrication, retention time
  - Decreased evaporation

https://onlinelibrary.wiley.com/doi/full/10.1111/aos.15675

Hyaluronic acid

D-glucuronic acid



N-acetyl-D-glucosamine

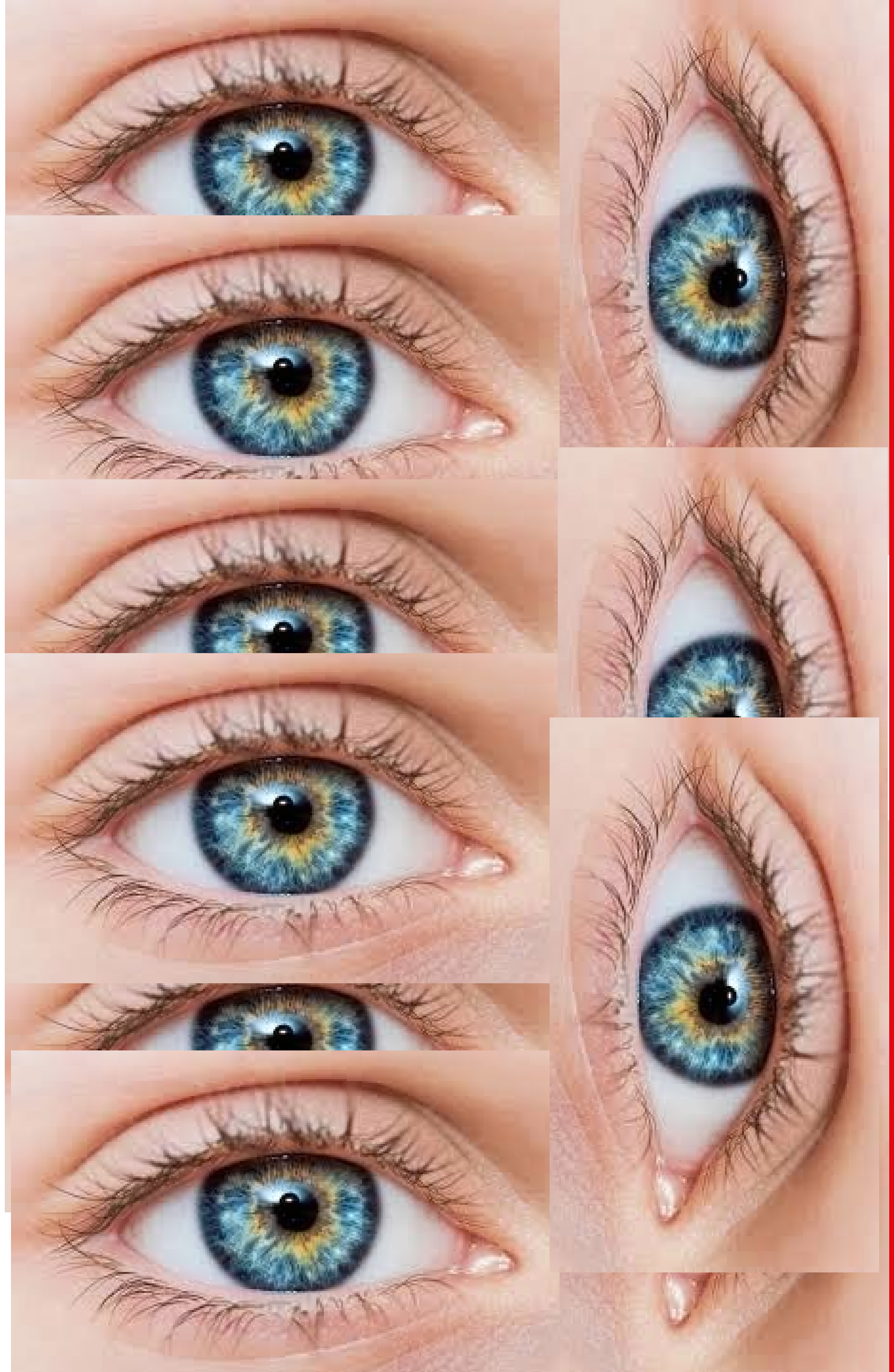
- Anionic, non sulfated, glycosaminoglycan
- Distributed widely throughout connective, epithelial, and neural tissues
- Used medically to treat osteoarthritis of the knee, wound healing, as a cosmetic filler, and to treat dry eye
- Described as "goo" molecule until late 1970s – ubiquitous polymer in ECM
- Role in lubrication and tissue repair, etc.,

https://onlinelibrary.wiley.com/doi/full/10.1111/aos.15675

Hyaluronic acid for dry eye

- Naturally occurring (including in vitreous humor, cornea, and tear film)
- Varying MW
- Properties for dry eye treatment: water retention and lubrication, reduces shear force on ocular surface, provides anti-inflammatory and antioxidant effects
- HA drops are often used as a control when assessing the safety and efficacy of new dry eye treatment options due to long history of safety and efficacy in opthamology

https://onlinelibrary.wiley.com/doi/full/10.1111/aos.15675
https://www.nature.com/articles/s41598-022-21330-0



Self-lubricating, Living Contact Lenses

Onto the paper…

ADVANCED MATERIALS

JOURNAL METRICS >
Online ISSN: 1521-4095
Print ISSN: 0935-9648
Editors-in-Chief: Irem Bayindir-Buchhalter, Esther Levy, Deputy Editors: Geraldine Echue, David Huesmann, Duoduo Liang, Sneha K Rhode, Bo Weng

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- 2023 IF = 27.4
- 2023 acceptance rate = 21%
- 2023 submission to first decision = 14 days
- 2023 submission to acceptance = 74 days

ADVANCED MATERIALS

www.advmat.de

Self-Lubricating, Living Contact Lenses

*María Puertas-Bartolomé, Izabook Gutiérrez-Urrutia, Lara Luana Teruel-Enrico, Cao Nguyen Duong, Krupansh Desai, Sara Trujillo, Christoph Wittmann, and Aránzazu del Campo**

The increasing prevalence of dry eye syndrome in aging and digital societies compromises long-term contact lens (CL) wear and forces users to regular eye drop instillation to alleviate discomfort. Here a novel approach with the potential to improve and extend the lubrication properties of CLs is presented. This is achieved by embedding lubricant-secreting biofactories within the CL material. The self-replenishable reservoirs autonomously produce and release hyaluronic acid (HA), a natural lubrication and wetting agent, long term. The hydrogel matrix regulates the growth of the biofactories and the HA production, and allows the diffusion of nutrients and HA for at least 3 weeks. The continuous release of HA sustainably reduces the friction coefficient of the CL surface. A self-lubricating CL prototype is presented, where the functional biofactories are contained in a functional ring at the lens periphery, outside of the vision area. The device is cytocompatible and fulfils physicochemical requirements of commercial CLs. The fabrication process is compatible with current manufacturing processes of CLs for vision correction. It is envisioned that the durable-by-design approach in living CL could enable long-term wear comfort for CL users and minimize the need for lubricating eye drops.

management, digital medicine, and communication.[1] The transfer of this vision into widely accepted user products demands CL materials with the highest and most durable comfort.[2] In soft CLs, user comfort depends on the capacity of the CL material to retain moisture, provide high lubrication, and avoid adsorption of tear fluid molecules on the CL surface, among other factors.[3] Wettability and water retention in CLs have been significantly improved in commercial CLs by grafting hydrophilic polymer layers, surfactants, or lipids on the surface of the lens (e.g., MoistureSeal, Aquaform or Hydraluxe technologies, among others).[4] Alternative academic approaches have been recently suggested based on embedded electronics[5] or engraved microchannels[6] to transport and retain tear fluid at the eye–lens interface.

RESEARCH ARTICLE

Editor's Choice

ADVANCED MATERIALS

www.advmat.de

Self-Lubricating, Living Contact Lenses

*María Puertas-Bartolomé, Izabook Gutiérrez-Urrutia, Lara Luana Teruel-Enrico, Cao Nguyen Duong, Krupansh Desai, Sara Trujillo, Christoph Wittmann, and Aránzazu del Campo**

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- Collaboration between research institute and university
- Two university departments: Chemistry and Systems Biotechnology
- Prof (del Campo) is doubly affiliated

https://www.leibniz-inm.de/en/about-inm/

https://www.uni-saarland.de/en/home.html

Dynamic Biomaterials

See! Research is fun!

We study and orchestrate how synthetic materials interact with living cells. We engineer cell-instructive environments and material-based solutions for zero-waste therapeutic solutions.

Our group develops hydrogel materials with programmed and tunable properties designed to encapsulate and instruct living cells. We study how living cells and inert matter interact and how these interactions can be exploited to direct cellular functions and ultimately result in therapeutic advantages. We cooperate with synthetic biologists, biophysicists, drug developers and clinicians to explore the application potential of our developments, with a focus on new materials for ophthalmic drug delivery. We contribute to INM's competence fields opto-interactive and bio-intelligent materials. Our research addresses biomedical needs.

- Interactions between synthetic materials and living cells
- Why? Therapeutic

Selected Publications

Actin-templated Structures: Nature's Way to Hierarchical Surface Patterns (Gecko's Setae as Case Study)

Kasper, Jennifer Y. | Laschke, Matthias W. | Koch, Marcus | Alibardi, Lorenzo | Magin, Thomas M. | Niessen, Carien M. | del Campo, Aránzazu

Advanced Science , 2024, 11 (10), 2303816.
https://onlinelibrary.wiley.com/doi/full/10.1002/advs.202303816

Discover more

Cytocompatibility evaluation of PEG-methylsulfone hydrogels

Trujillo, Sara | Kasper, Jennifer Y. | Miguel-Jimenez, Adrián De | Abt, Britta | Bauer, Alina | Mekontso Ngaffo, Joelle A. | Pearson, Samuel | del Campo, Aránzazu

ACS Omega , 2023, 8 (35), 32043-32052.
https://pubs.acs.org/doi/10.1021/acsomega.3c03952

Discover more

Rheological behavior of Pluronic/Pluronic diacrylate hydrogels used for bacteria encapsulation in engineered living materials

Bhusari, Shardul | Hoffmann, Maxi | Herbeck-Engel, Petra | Sankaran, Shrikrishnan | Wilhelm, Manfred | del Campo, Aránzazu

Soft Matter , 2023, 20 (6), 1320-1332.
https://pubs.rsc.org/en/Content/ArticleLanding/2024/SM/D3SM01119D

Discover more

Living contact lenses – abstract/introduction

Why even do this study?

- Dry eye syndrome! Oh non! (It’s actually serious)
- Compromises long term-contact lens wear
- Users require eye drops to alleviate discomfort

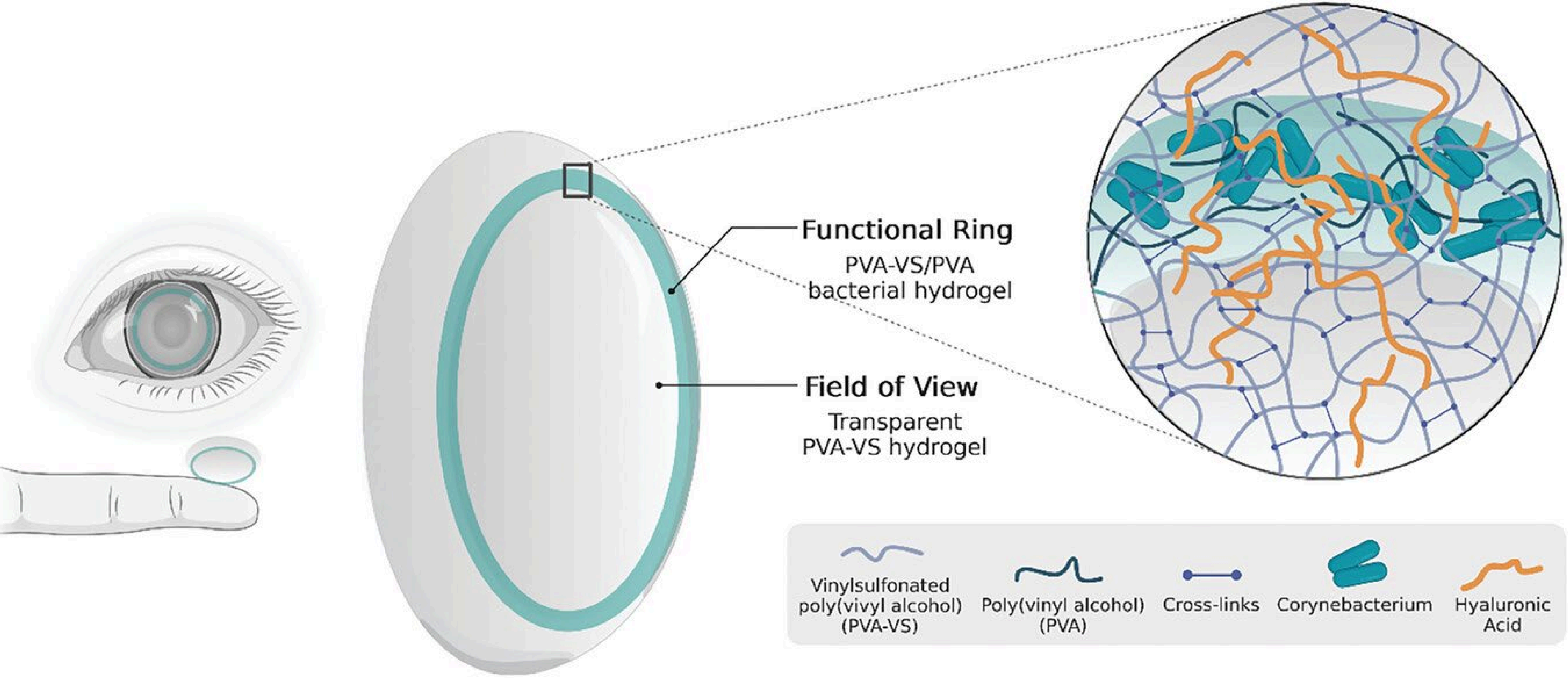
Living contact lenses – abstract/introduction

Hint!
Biofactory = bacteria

So what did they do?

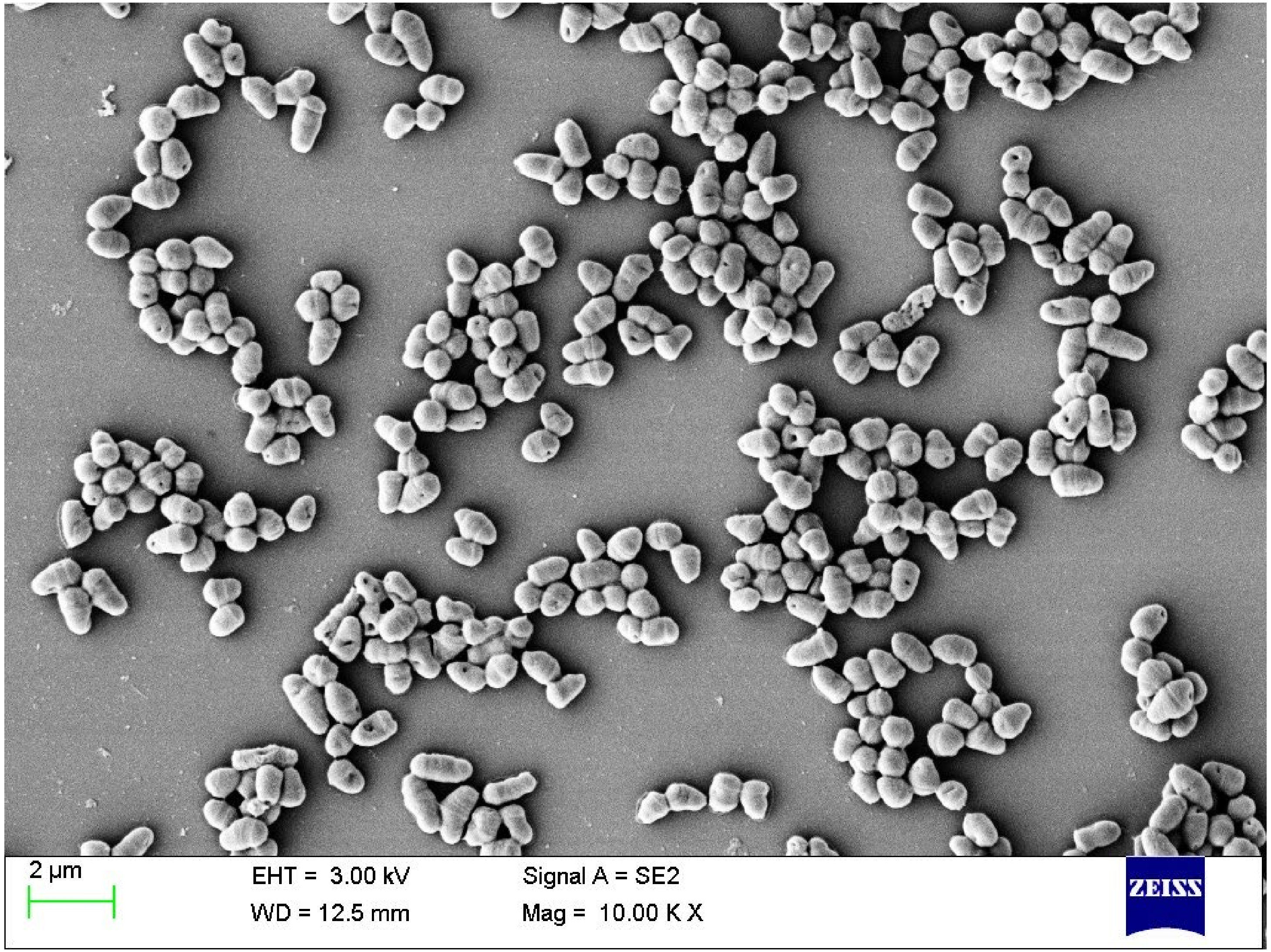
- Embedded a lubricant secreting “biofactory” with the CL
- Self-replenishable
- Hydrogel matrix regulates the growth of the “biofactories” and the HA production, and allows the diffusion of nutrients and HA for at least 3 weeks
- Continuous release of HA reduces the friction coefficient of the CL surface
- prototype is presented, where the biofactories are contained in a functional ring at the lens periphery, outside of the vision area

Living contact lenses – abstract/introduction



Living contact lenses – results

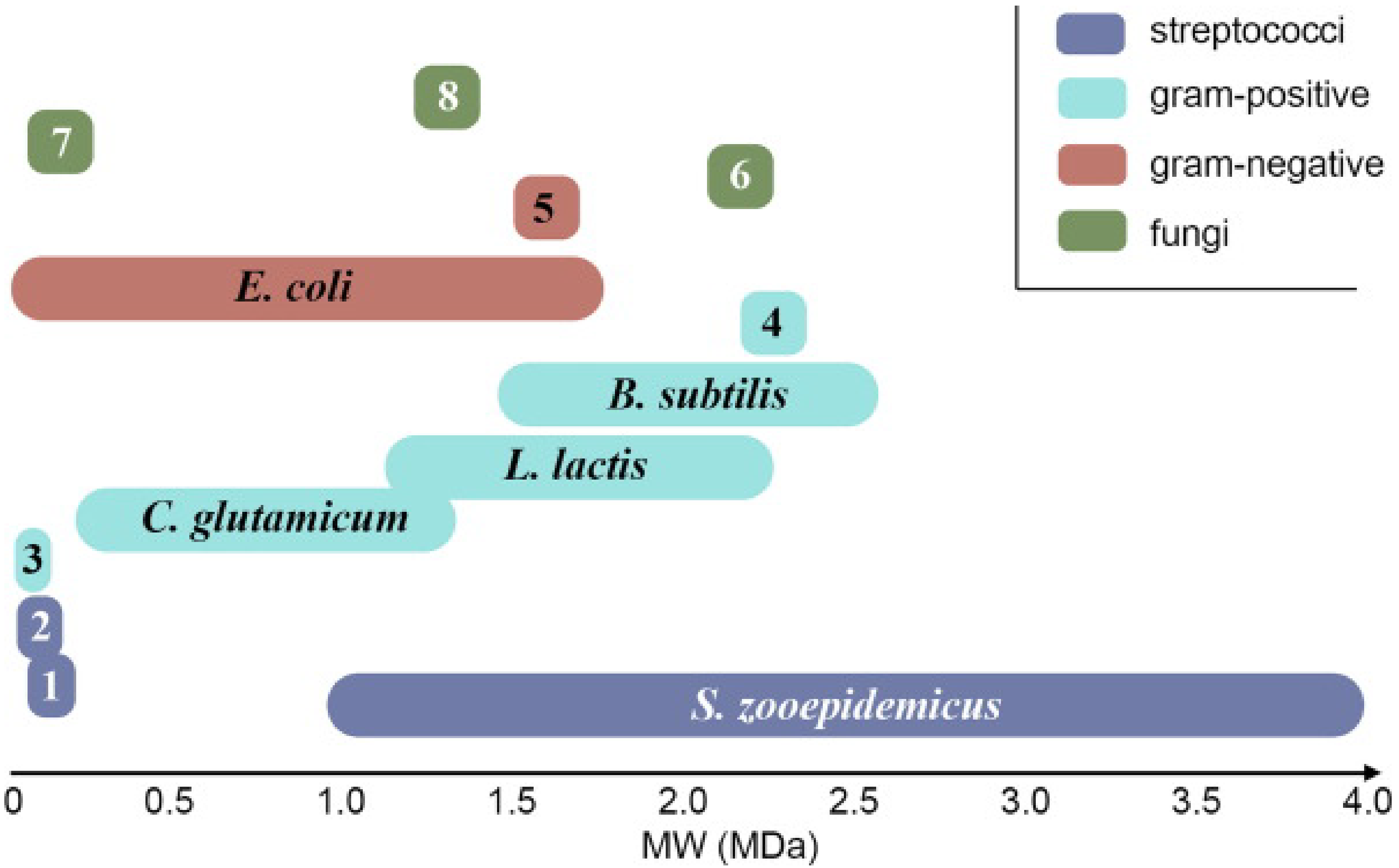
[C. glutamicum](#) [Prospective bacterial and fungal sources of HA_2022](#)



- *Corynebacterium glutamicum*
- Gram-positive
- Rod-shaped
- Used for large scale production of various organic chemicals, such as organic acids, alcohols, amino acids
- No exdotoxins
- GRAS

HA MWs produced from various sources

Prospective bacterial and fungal sources of HA_2022



GRAS = Generally recognized as safe

"GRAS" is an acronym for the phrase **G**enerally **R**ecognized **A**s **S**afe. Under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (the Act), any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excepted from the definition of a food additive.

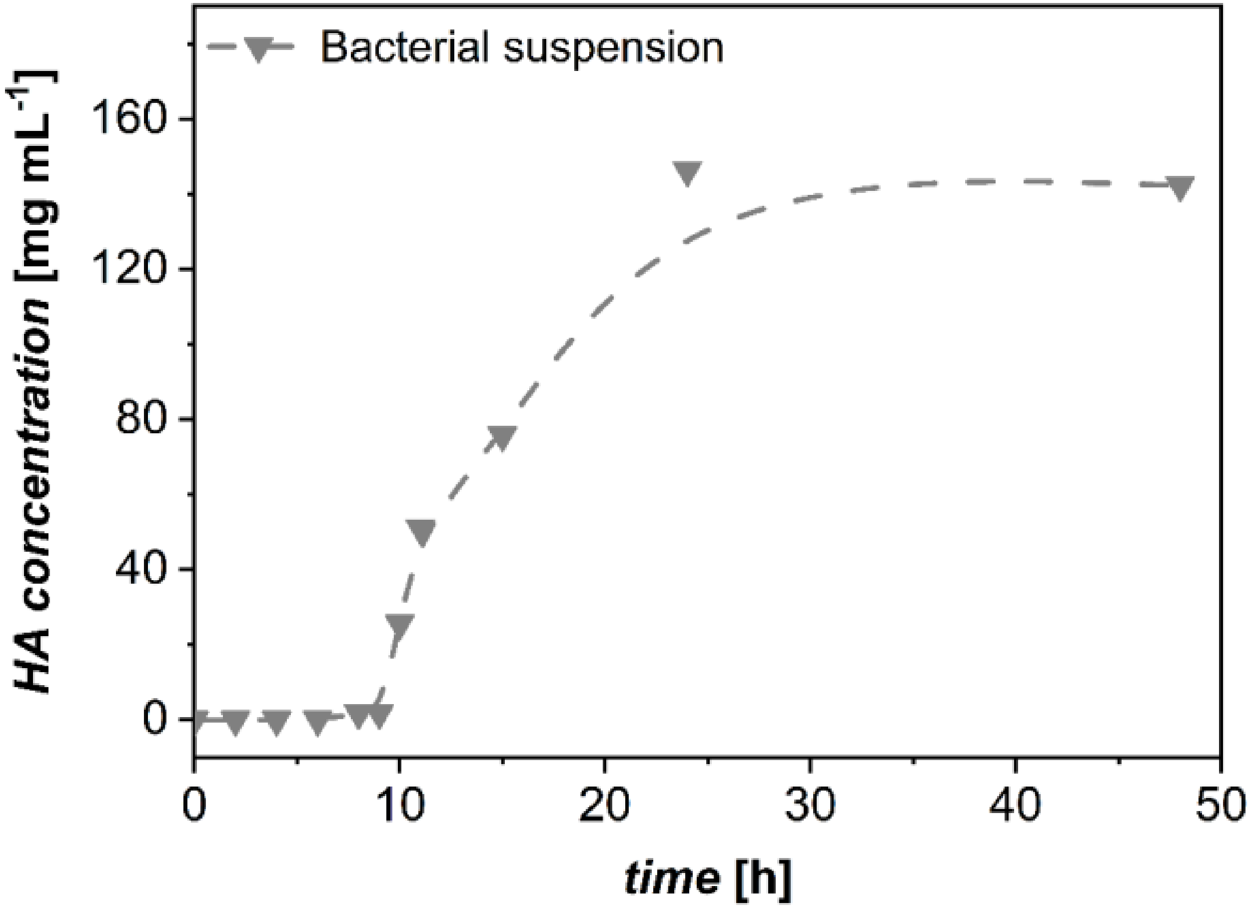
https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras

Living contact lenses – the bacteria

- *C. glutamicum* DSM 20300
- Wild type genetically engineered to secrete HA
- Step 1: eliminate native *ldhA* gene encoding lactate dehydrogenase to prevent accumulation and secretion of lactate under oxygen-limiting conditions (mutant *C. glutamicum ΔldhA)*
- Step 2: expressed the two-gene biosynthetic cluster *hasAB* from *Streptococcus equisimilis*, encoding HA synthetase and UDP-glucose dehydrogenase, respectively, in the mutant to enable HA production

Breaking it down:

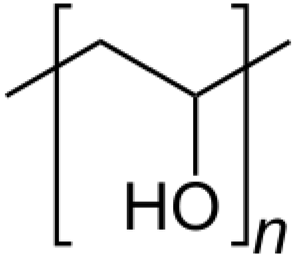
- *hasA* makes the enzyme that produces HA (HA synthetase)
- *hasB* makes the enzyme that creates one of the HA building blocks (glucuronic acid)
- Promoter *Ptuf* is a constitutive promoter, upstream of hasAB; constitutive means that the gene is turned on all the time, for continuous HA production
- ON switch (*P*tuf) → gene 1 (*hasA*) → gene 2 (*hasB*)



- Showing that HA is produced in suspension culture
- Seems to need about 10 h

Figure S1: Cumulative HA concentration released in the medium from suspension culture of the engineered HA producer *C. glutamicum*. No medium change was performed during the incubation time. HA concentration was measured by GPC analysis of the supernatant.

Living contact lenses – the hydrogel



PVAc Ethanol

$$[CH_2CH(OAc)]_n + C_2H_5OH \rightarrow [CH_2CH(OH)]_n + C_2H_5OAc$$

PVA Ethyl acetate

- Based on polyvinyl alcohol
- Water soluble
- Synthetic
- Highly used in different areas
- Biocompatible hydrogels, also used in contact lenses

- Not produced by monomer polymerization – monomer vinyl alcohol is thermodynamically unstable
- Produced by the hydrolysis of polyvinyl acetate
- Properties defined by MW and degree of deacetylation
- More hydrolysis = water soluble; and vice versa

PVA or PVOH

Living contact lenses – the hydrogel

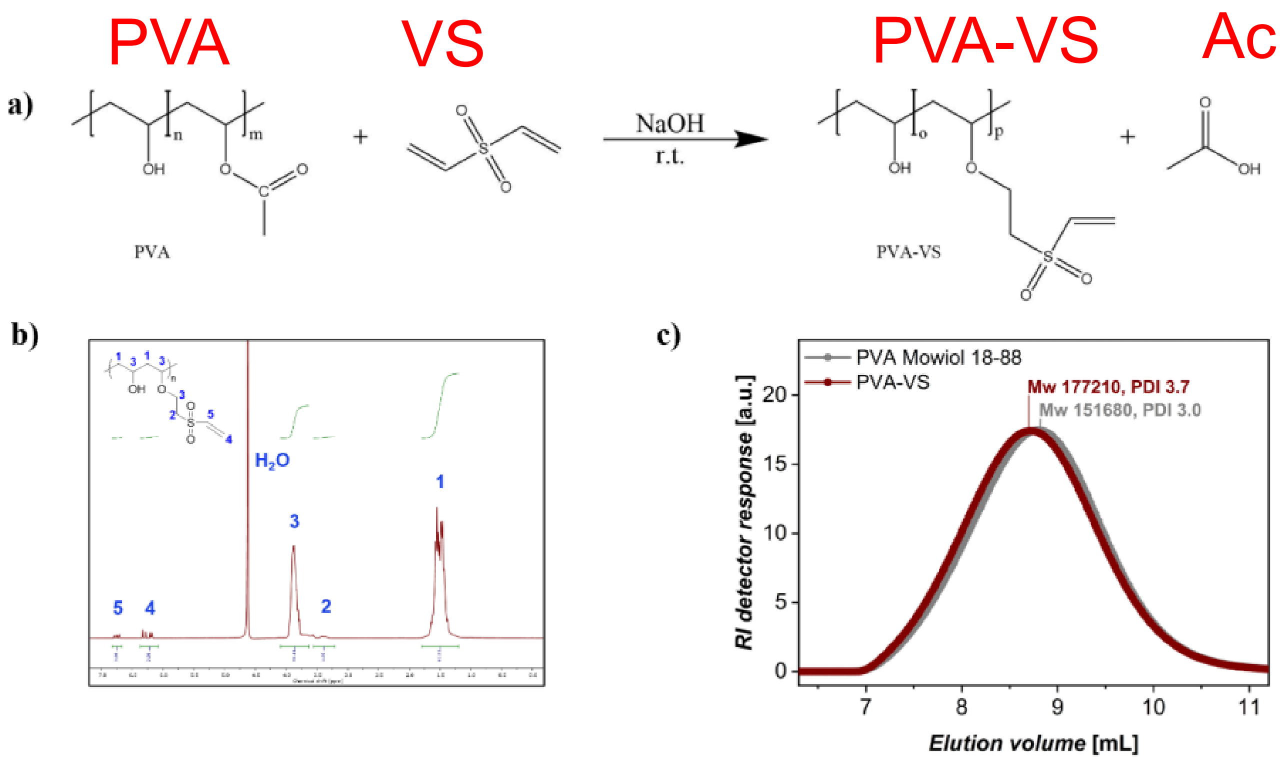


Figure S2: a) Chemical functionalization of PVA with divinylsulfone (DS) to form vinyl sulfonated poly(vinyl alcohol) (PVA-VS). **b)** ^{1}H-NMR spectrum of PVA-VS in D_2O. The integrals of the vinyl signals indicate a 1.5 ±0.2% degree of VS modification. **c)** GPC elugram of PVA (Mowiol 18-88) and PVA-VS showing no significant changes in the molecular weight distribution after the VS-functionalization reaction.

- Hydrogels contained PVA and modified PVA-vinyl sulfone (VS)
- Is PVA-VS ok for contact lenses?
- PVA-VS can be crosslinked with light with LAP as photoinitiator
- Non-crosslinkable PVA is included to modulate the porosity of the hydrogels
- Screening results: 5-10% w/v PVA-VS with 1.5% degree of VS functionalization gives mechanically stable and transparent hydrogels
- Bacteria could grown in these gels (data not shown)

Living contact lenses – the hydrogel

Table 1. Physicochemical properties of PVA-VS/PVA hydrogels and the commercial PVA-based Focus Dailies CL (Alcon) measured in STF.

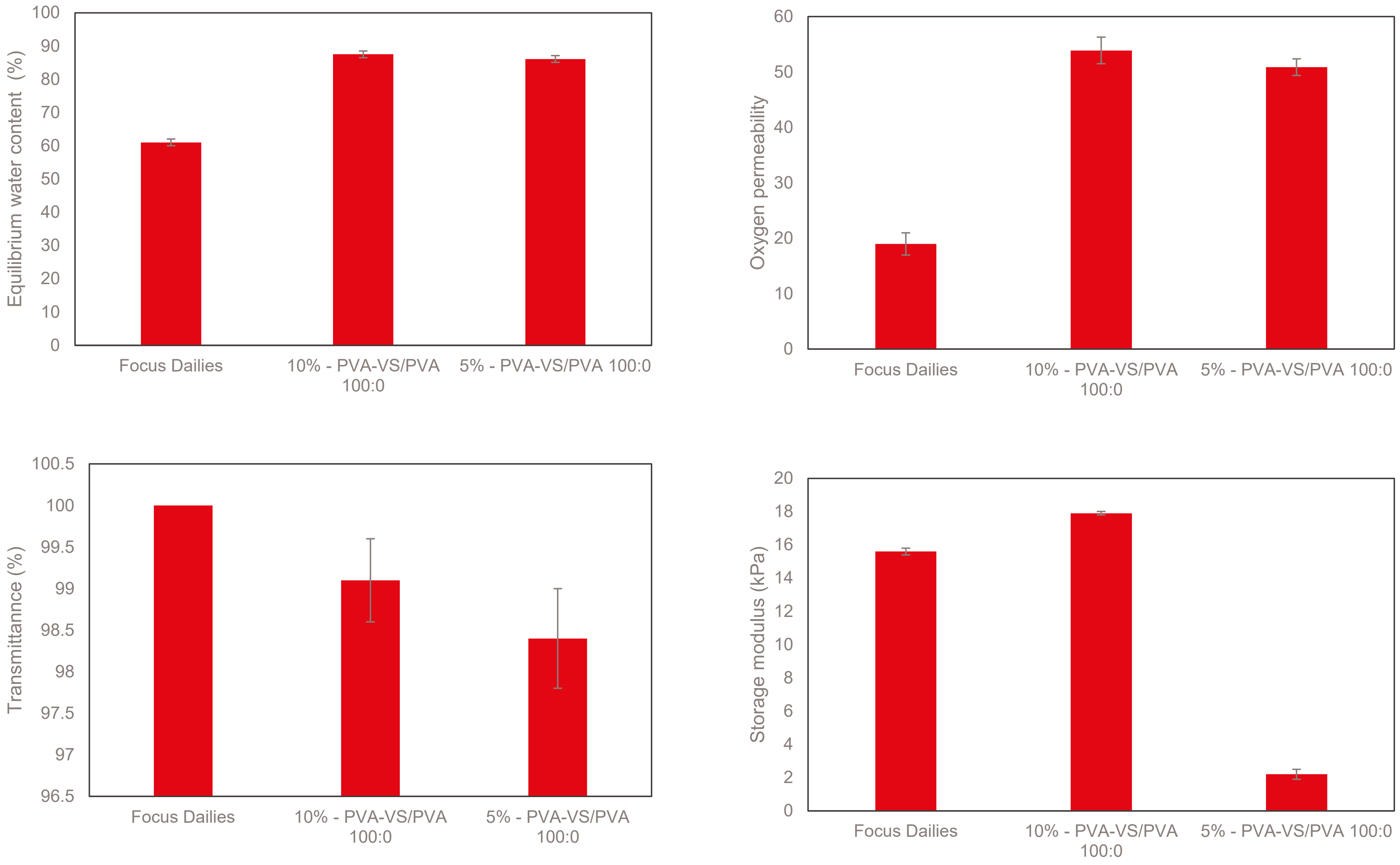
Material	Polymer content [% w/v]	Equilibrium water content (EWC) [%]	Oxygen permeability D_k [barriers][a)]	Water contact angle [°]	Transmittance [%][b)]	Refractive index	Storage modulus G' [kPa][c)]
Focus Dailies	–	61 ± 3	19 ± 2	28 ± 4	>99	1.380	15.6 ± 0.2
PVA-VS/PVA 100:0	10	87.5 ± 0.9	53.9 ± 2.4	29 ± 3	99.1 ± 0.5	1.336	17.9 ± 0.1
PVA-VS/PVA 100:0	5	86.1 ± 0.6	50.9 ± 1.5	23 ± 7	98.4 ± 0.6	1.334	2.2 ± 0.3
PVA-VS/PVA 99:1	5	86.6 ± 1.1	52.1 ± 2.8	22 ± 3	98.4 ± 0.3	1.333	1.6 ± 0.2
PVA-VS/PVA 95:5	5	85.3 ± 0.5	49.4 ± 1.1	23 ± 1	98.7 ± 0.8	1.334	1.5 ± 0.1

[a)] Calculated from EWC measurements; [b] Value at 600 nm; [c)] Obtained from time sweep measurements.

- [a)] Calculated from EWC measurements;
- [b] Value at 600 nm;
- [c)] Obtained from time sweep measurements.

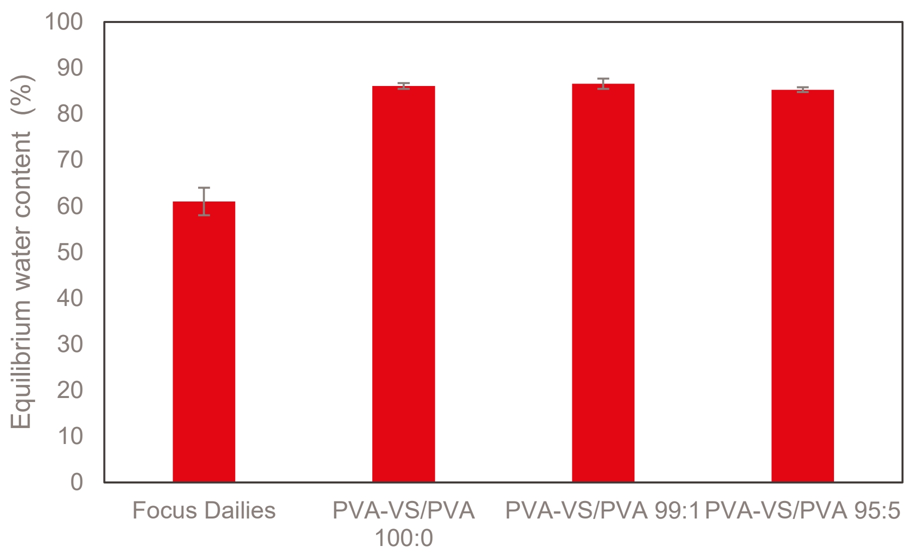
Big table of properties, not super visual – can I make it clearer?

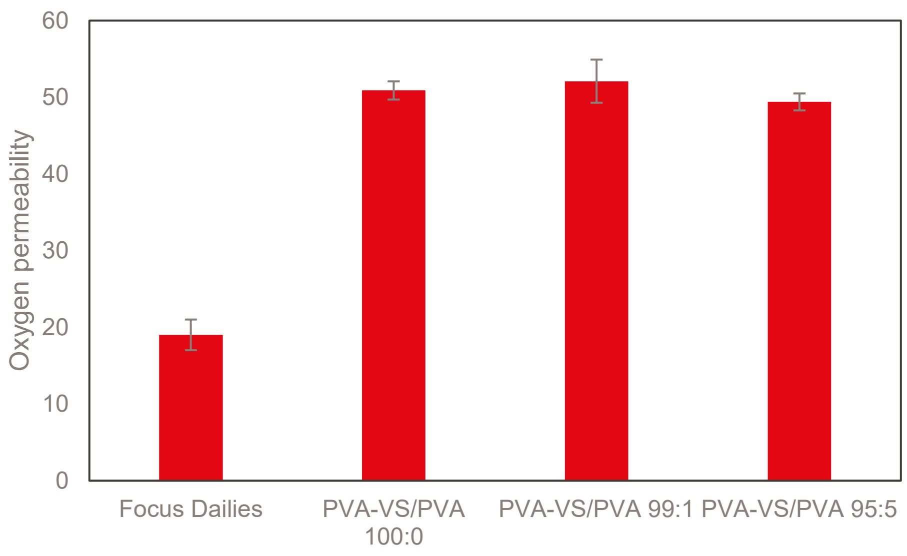
Living contact lenses – effect of polymer content

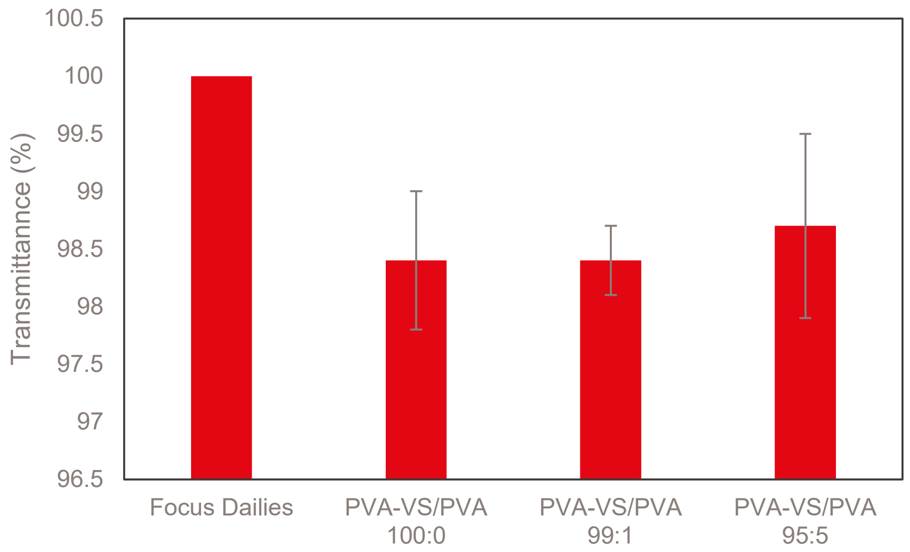


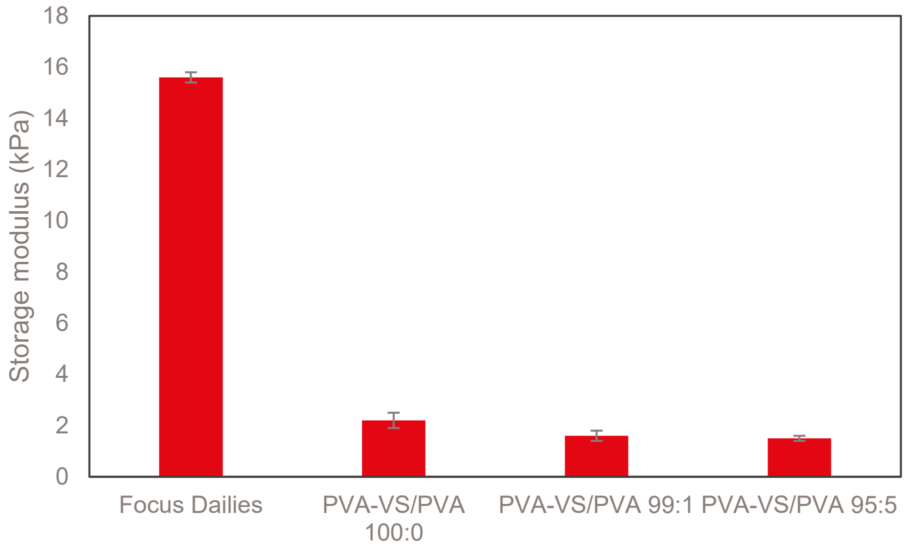
- Only see effect of 5 vs.10% in G'

Living contact lenses – effect of free PVA



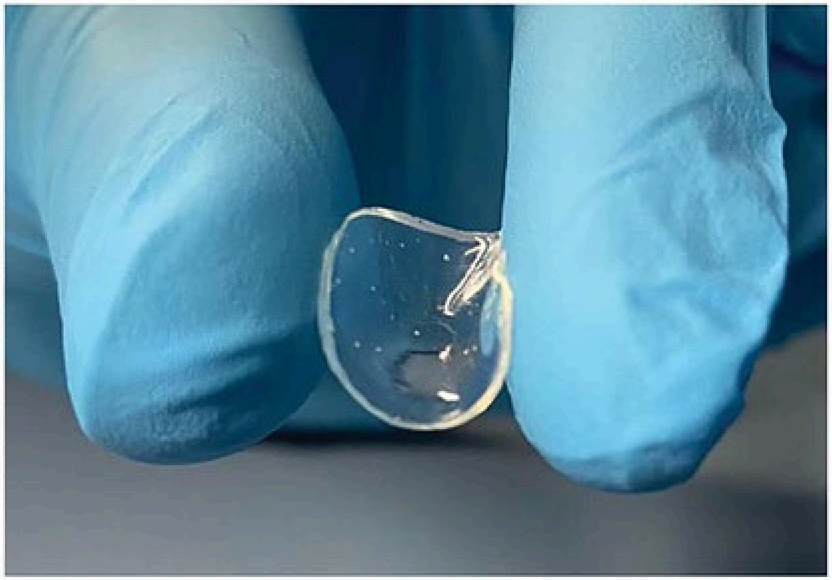






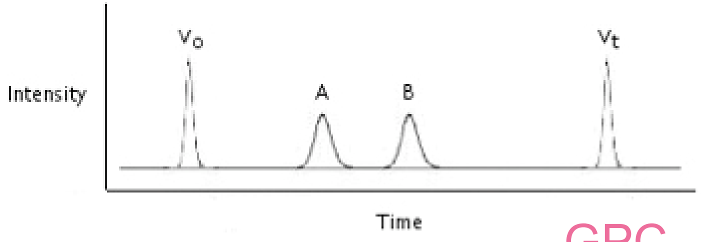
- All at 5%
- No big diff
- Not exactly tunable…

Transparent & soft lenses - 5% free PVA



- PVA-VS/PVA 100:0 hydrogel discs
- Scale = 1 cm

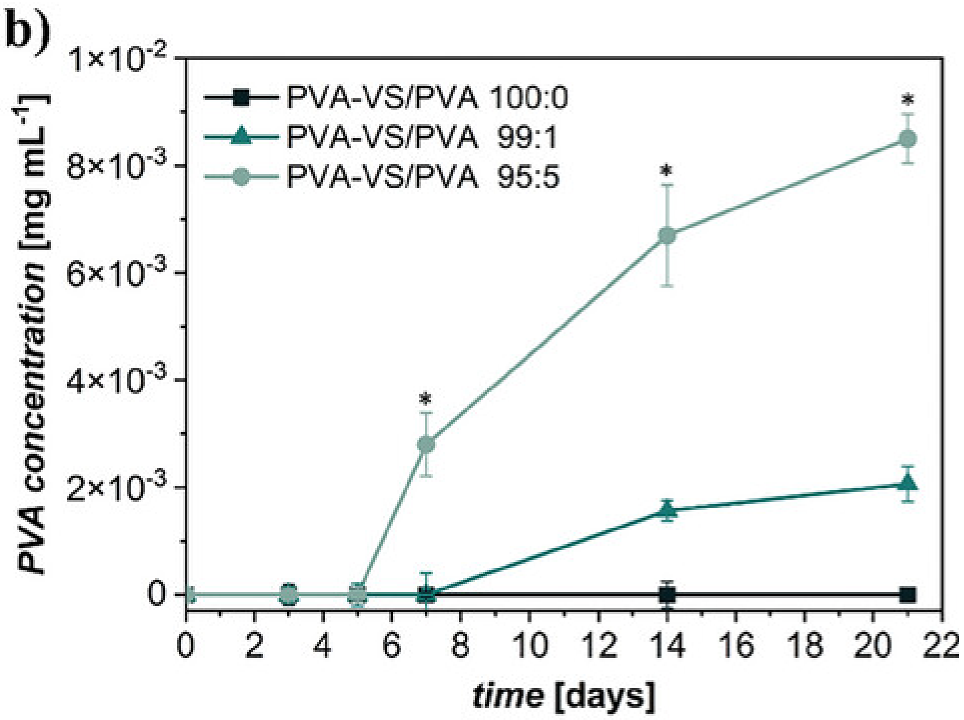
PVA leaching

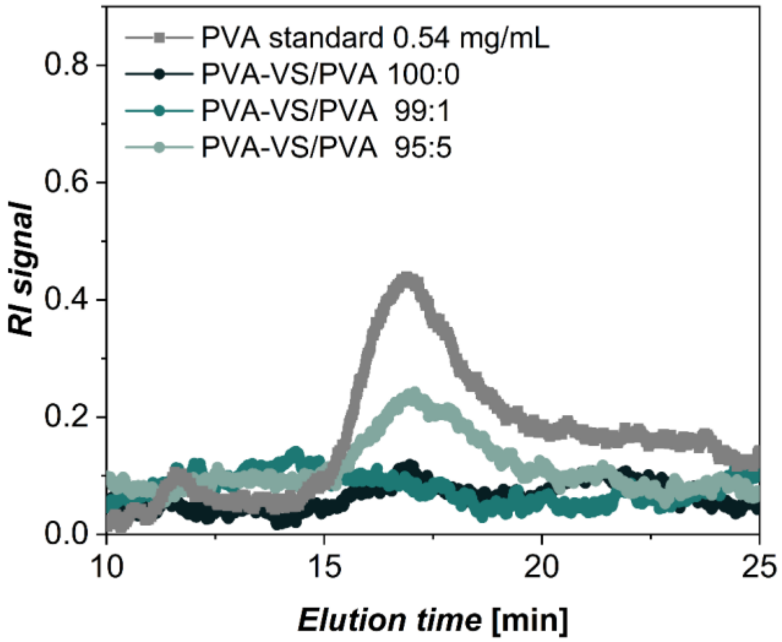


GPC

PVA release in STF at 30 °C for 3, 5, 7, 14, and 21 days

GPC (gel permeation chromatography) of supernatant

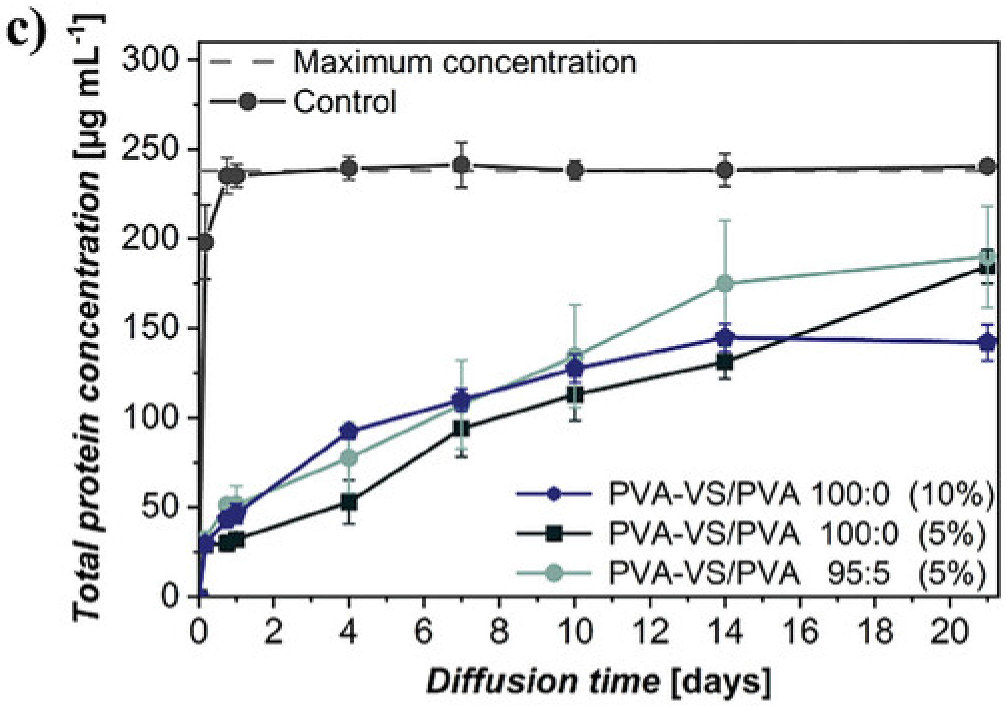


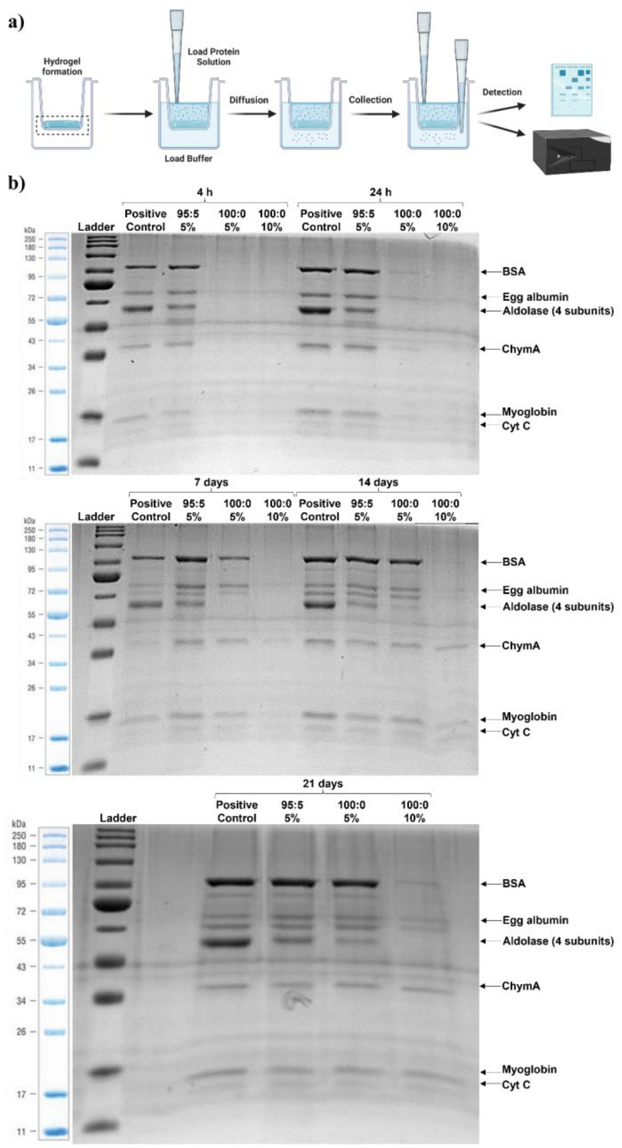


- No PVA release in 100-0; all xlinked
- <25% PVA loss in 1% and 5% free PVA - mostly entrapped

Protein diffusion through hydrogels – 12.3-160 kDa mixture

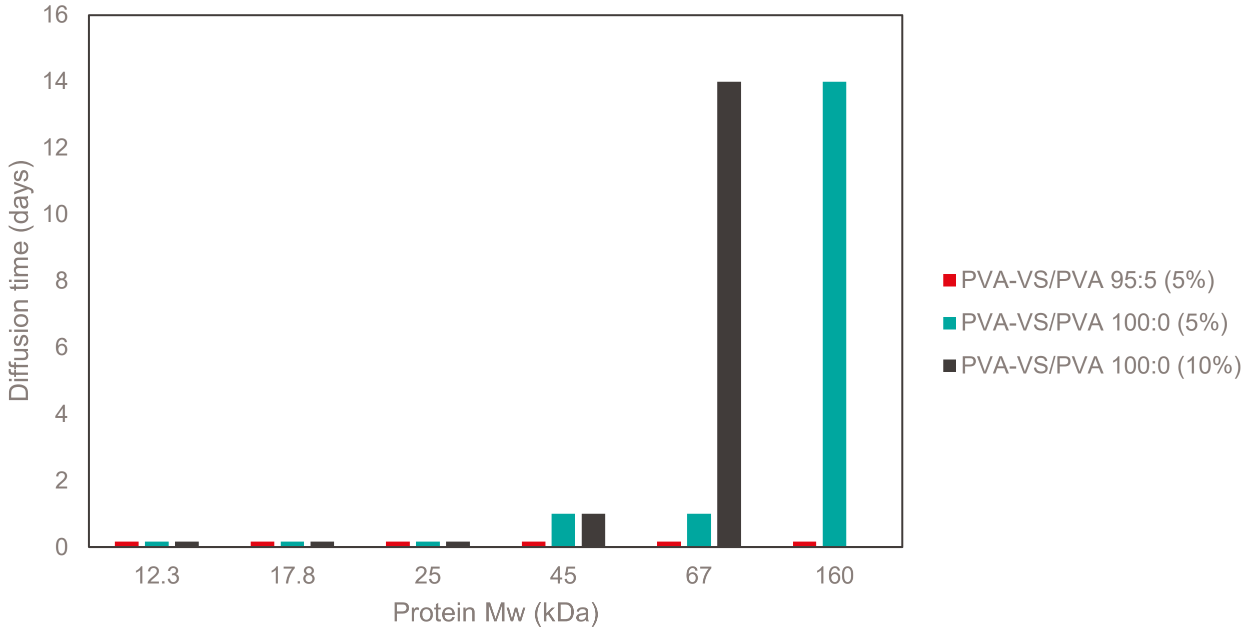
BCA assay





- Used BCA assay to detect total protein concentration in supernatant

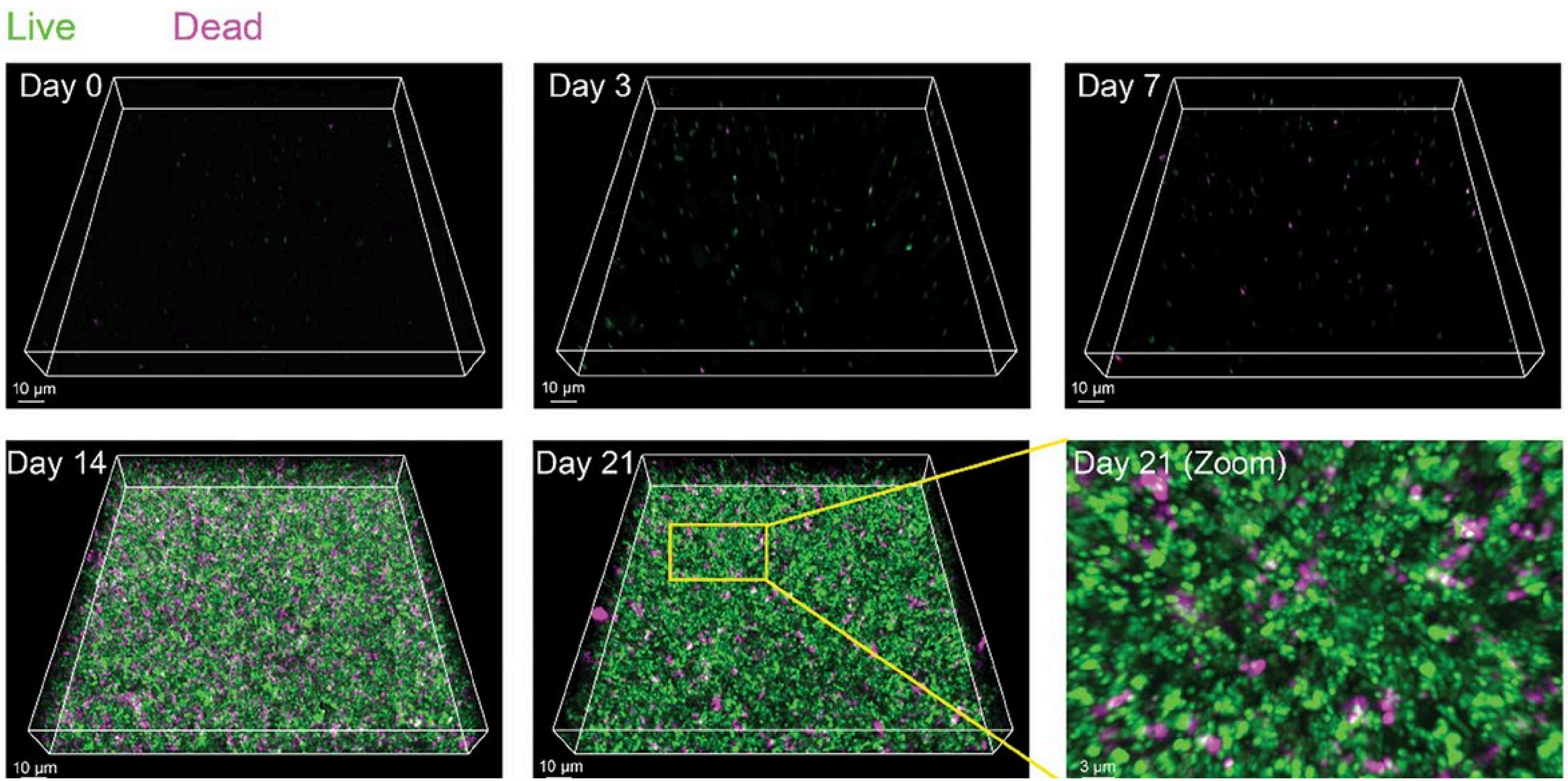
Porosity of hydrogels by diffusion



- All proteins were able to diffuse through the 5% w/v hydrogels, indicating that the pores of the network are larger than 160 kDa
- Proteins with molecular weight <25 kDa diffused through the hydrogels within 4 h
- Proteins with size >25 kDa required 4 h to diffuse through the hydrogel that included non-crosslinked PVA, and 1 to 14 days when the PVA-VS content increased from 5% to 10% w/v

EPFL

Living hydrogel – 5% w/v PVA-VS/PVA 95:5



- *C. Glutamicum* mixed with hydrogel precursor solution
- Photo crosslinking in mold
- Contact time of bacteria and precursors <1 min (177 kDa polymers not expected to penetrate Gram positive bacteria in this time scale)
- Expect that the bacteria is entrapped not crosslinked

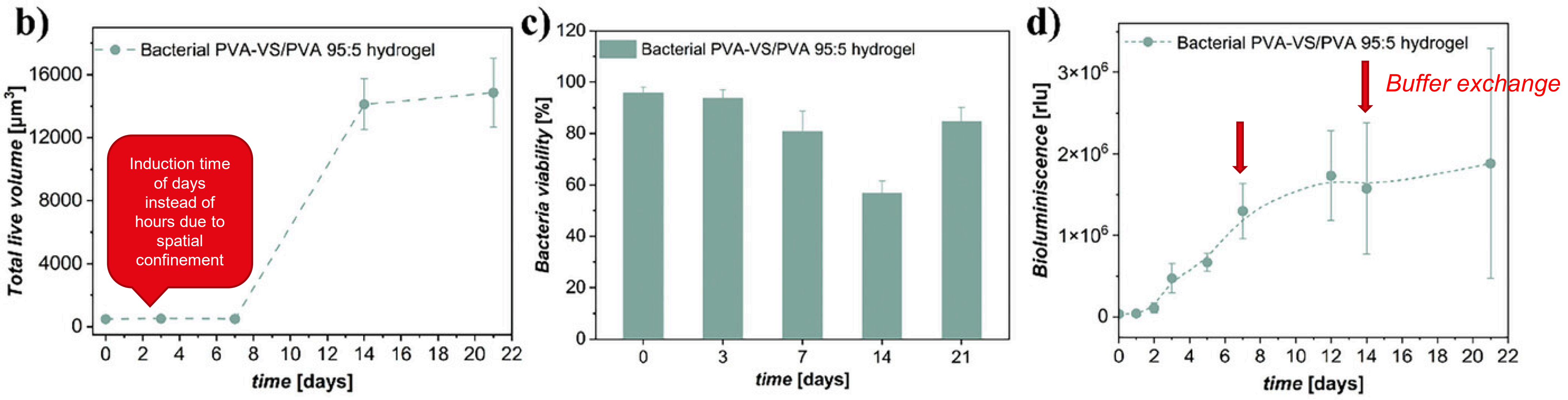
- Growth of bacteria in the hydrogel during incubation in medium for 21 days at 30 °C was monitored by fluorescence imaging after Live/Dead staining (time scale relevant to real life CL use –weekly/monthly)

Living hydrogel - 5% w/v PVA-VS/PVA 95:5

From Live/Dead

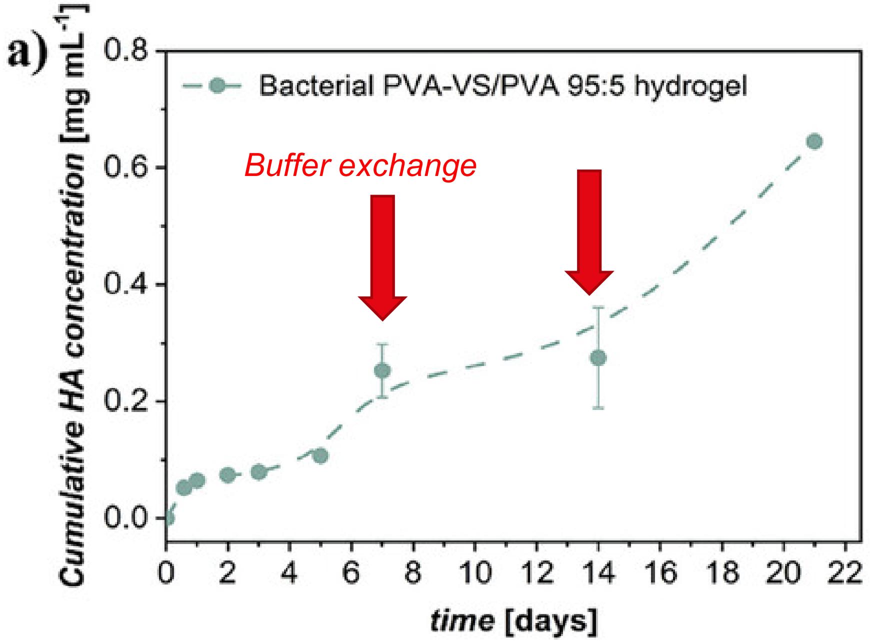
From Live/Dead

From 3D bioluminescence assay



- Estimates by image analysis show induction, growth, and steady-state over several days (b)
- Viability was high over 21 days (a, c)
- Metabolic activity by quantifying ATP in hydrogels using a 3D viability assay (d) – indicates an increase in metabolic activity during the first week followed by a constant value at longer times
- Conclusion – hydrogel system supports the spatially contained growth and activity of the bacteria for at least 3 weeks

Release of HA from living hydrogel - 5% w/v PVA-VS/PVA 95:5



- GPC of supernatant
- HA with a MW of 30-70 kDa was detected after 14 h
- *C. glutamicum* in PVA-VS hydrogels to produce and release HA

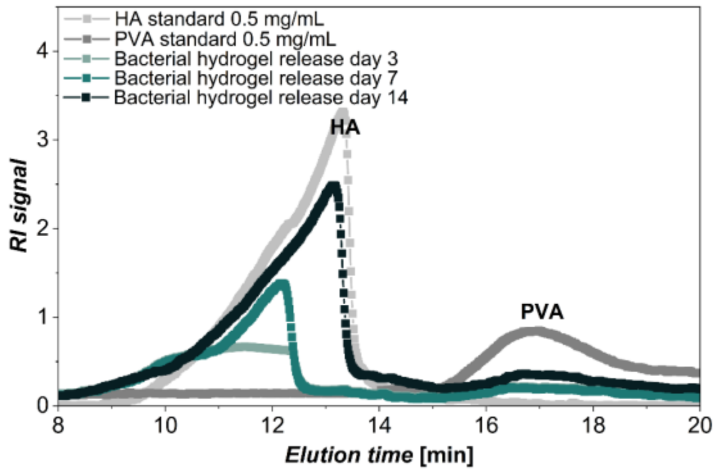
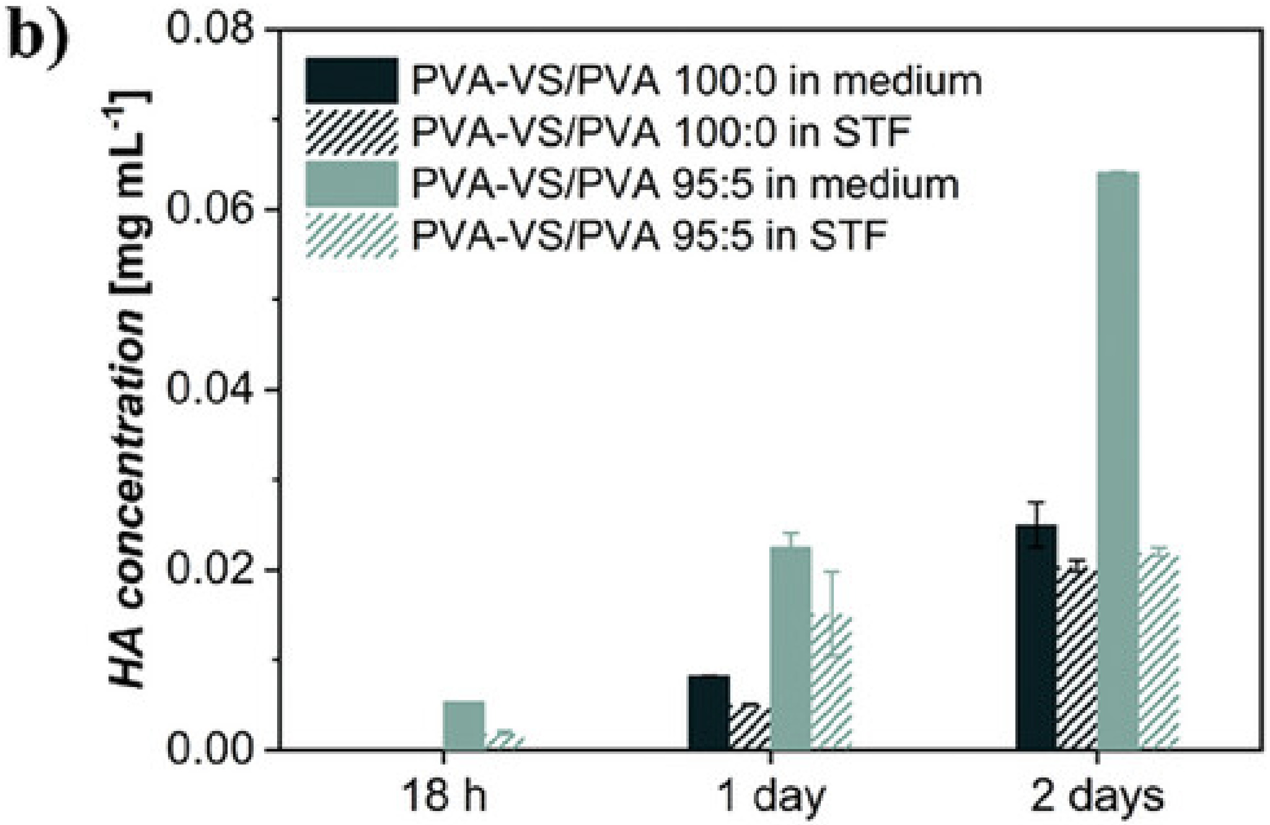


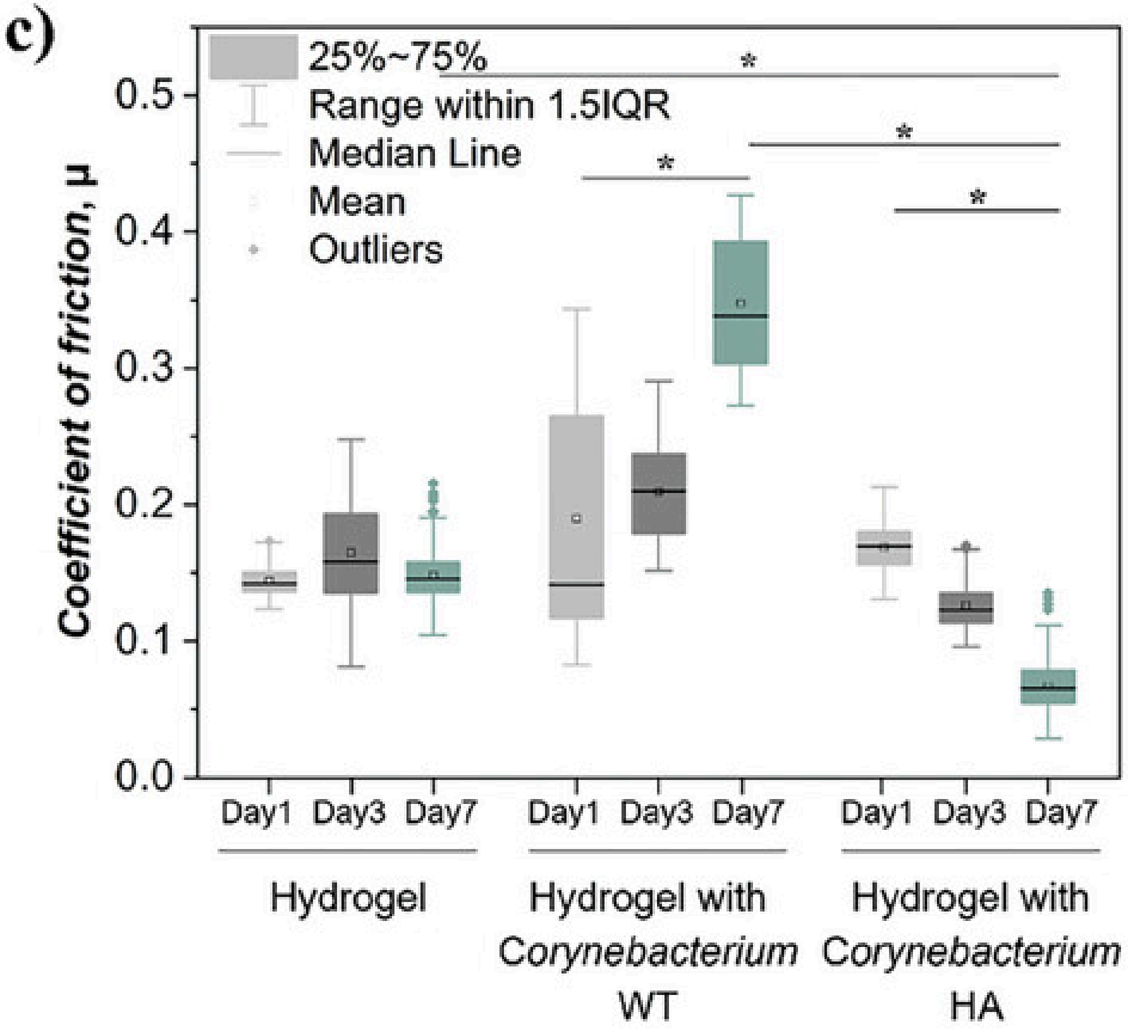
Figure S10. Representative GPC chromatograms of supernatants of PVA-VS/PVA 95:5 bacterial hydrogels after 3, 7, and 14 days of incubation. The chromatograms of standard HA (0.5 mg/mL) and PVA (0.5 mg/mL) solutions are also shown.

Release of HA from living hydrogel - 5% w/v PVA-VS/PVA 95:5



- GPC of supernatant
- More production of SA in medium than in STF (simulated tear fluid)
- Addition of non-crosslinked PVA, increases rerelease of HA, attributed to greater growth of bacteria and faster diffusion in less crosslinked media

Self-lubrication properties of living hydrogel

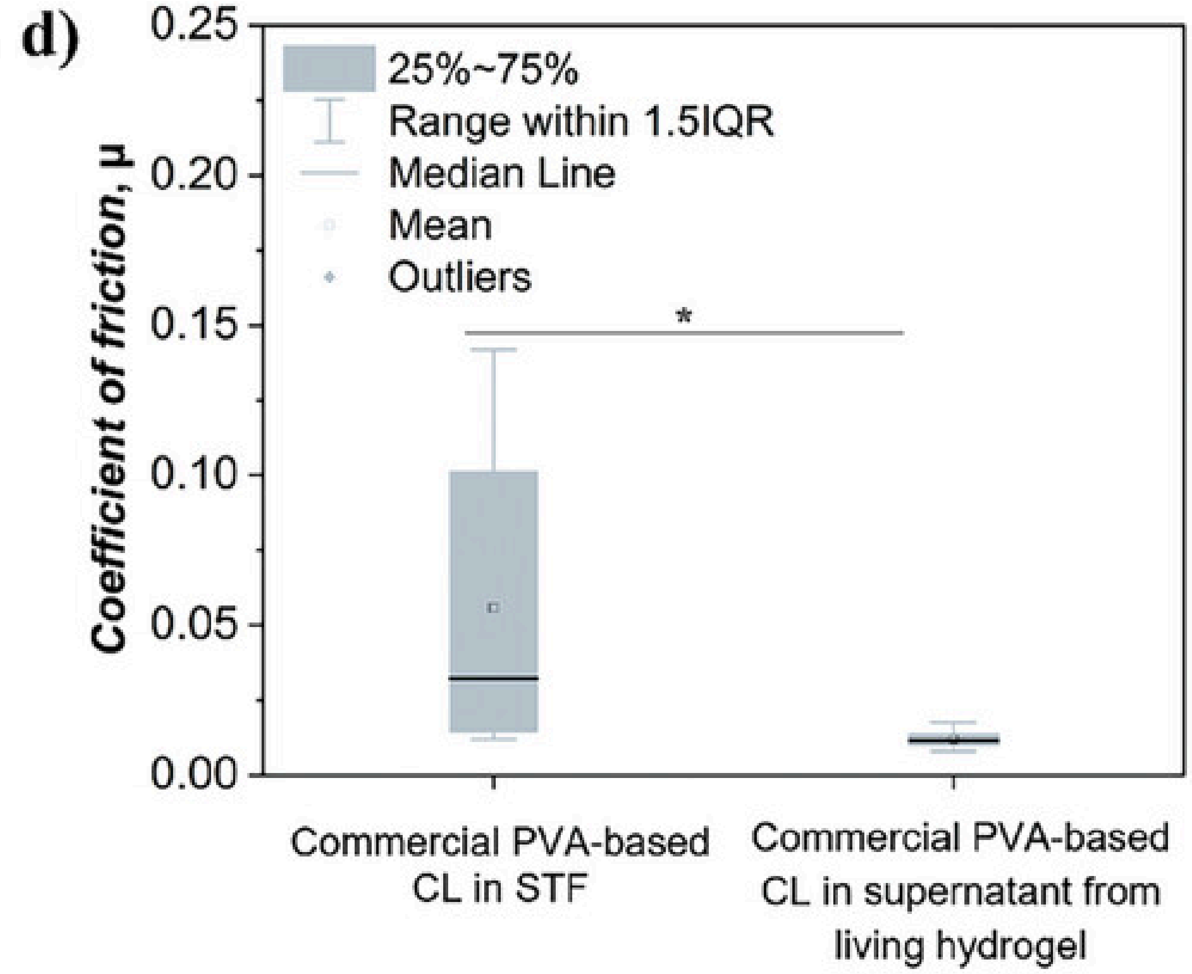


- 5% w/v PVA-VS/PVA 95:5
- Control is WT which does not produce HA and empty hydrogel
- Hydrogel disk attached to upper plate of rheometer and torque measured against steel bottom plate in the presence of the corresponding supernatant while an angular for is applied

- WT show an increase in friction coefficient, attributed to the release of metabolites and proteins that can interact with the hydrogel

- Opposite is seen for HA producing hydrogels! Self-lubrication!

Self-lubrication properties of living hydrogel

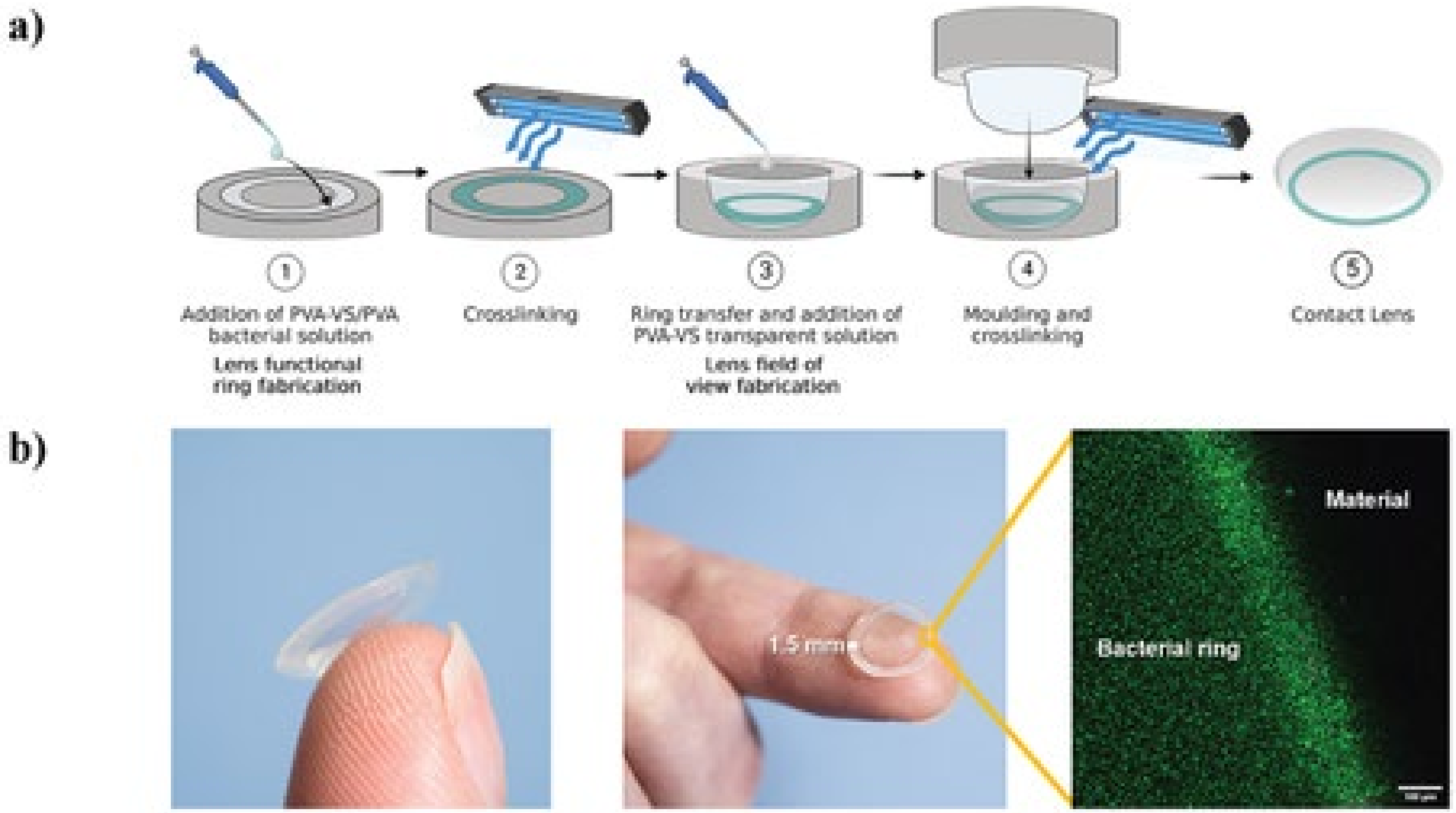


- Compared to commercial lenses
- Measure in supernatant after 7 days
- Also in this case, the friction coefficient of the CL surface significantly decreased when measured in supernatant, corroborating the lubricating properties of the secreted molecules/HA

Self-lubricating CL prototype

Why a ring?

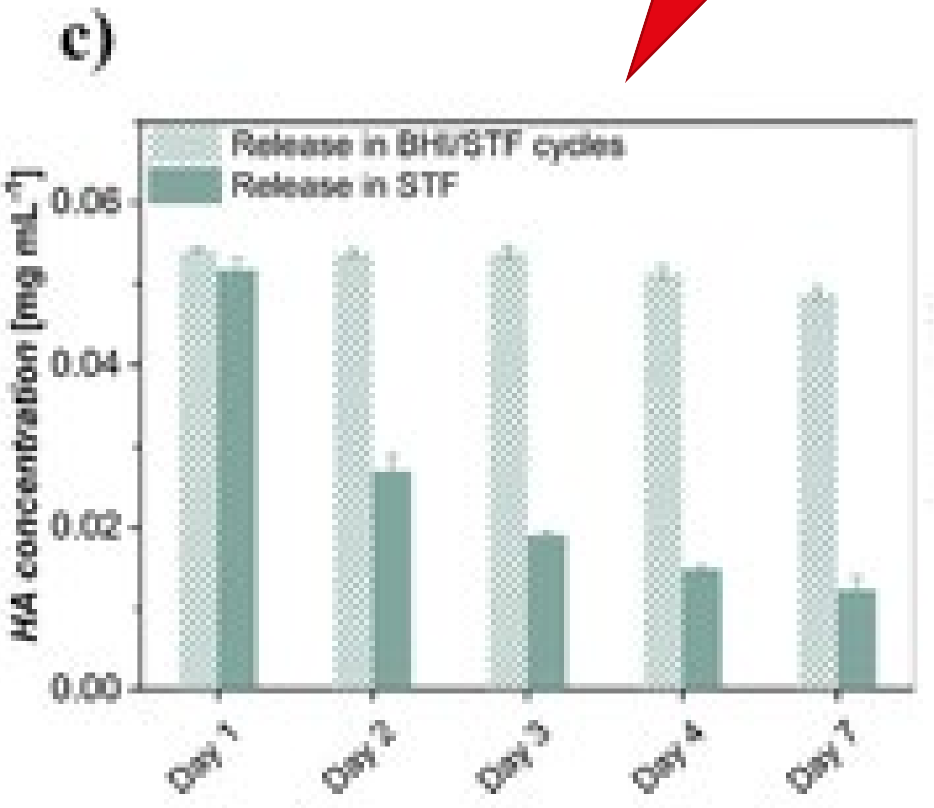
Why the different hydrogel formulations?



- 1st mold 5% hydrogel (free PVA) containing bacteria in ring (10 mm diameter)
- 2nd embed ring in 10 % hydrogel (no free PVA)
- 11 mm diameter, 1.5 mm thickness
- When incubated in medium, bacteria grew within ring, no outgrowth or leaking of bacteria

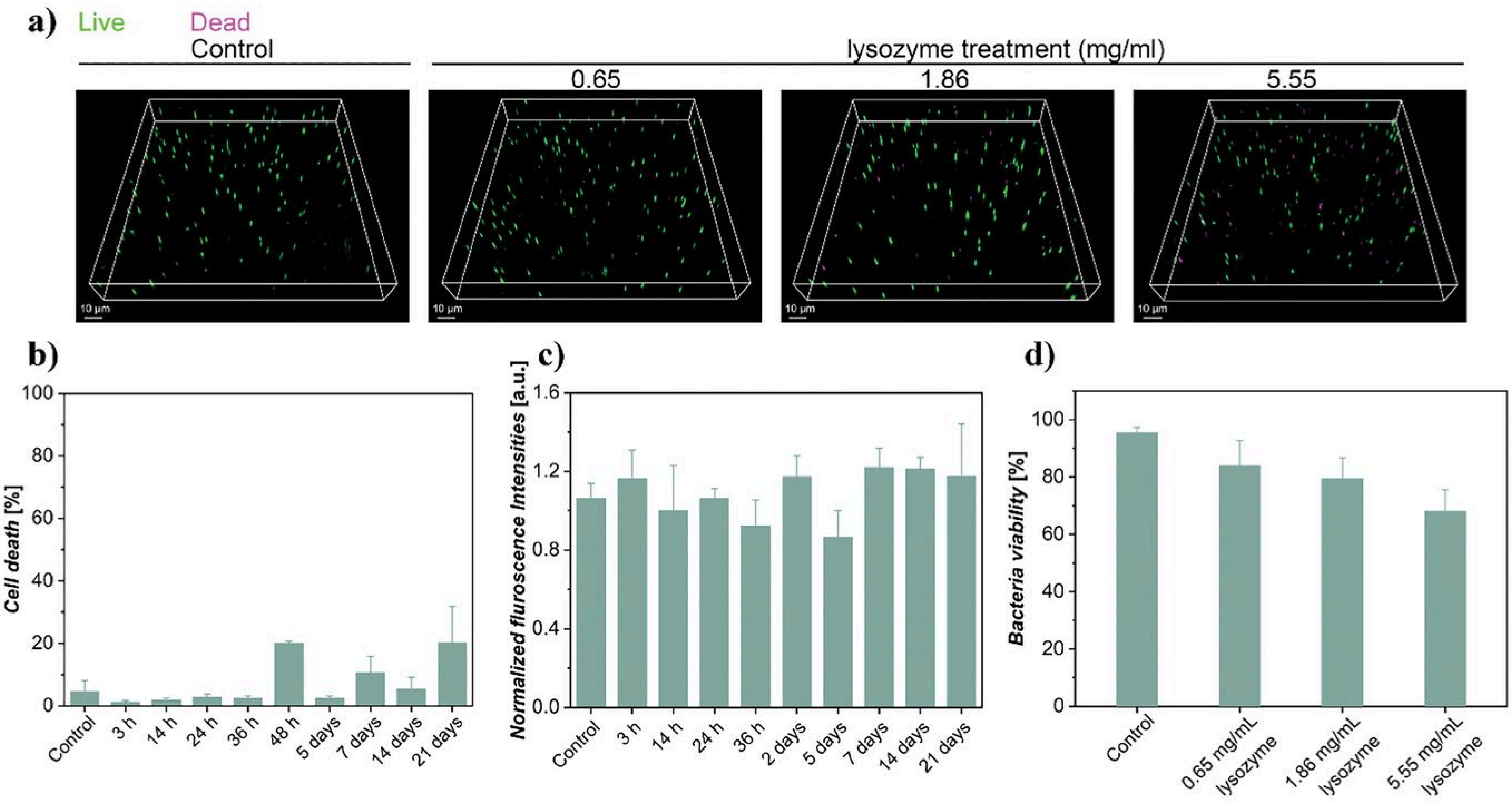
Self-lubricating CL prototype

Sustained release of a therapeutic!



- Release measured by supernatant GPC in media
- 1st case: Release in STF over days, measured after 24 hours
- 2nd case: cyclic 16 h in media, and 8 hours in STF
- In the first case, HA decreases over time due to lack of nutrients in medium
- In second case, constant release of HA over 7 days due to exposure to nutrients which sustains HA production
- Scenario – overnight incubation (16 h), daily release (8 h) = dailies!

Cytocompatibility with mouse fibroblastic cells



Conclusions

Why say bacteria? It's a biofactory!

- Self-lubricating living CL based on "embedded biofactories programmed to continuously produce and release HA", a natural lubricant

- Based on PVA-VS; at 5% this hydrogel can be mixed with "biofactories", and aftyer crosslinking it supports viability and metabolic activity for at least 3 weeks

- 7 day HA release in tunable and sustained manner

- Interesting for future CL applications, but more studies needed to fully understand control/release profiles, etc.,

Did you like this paper? Why or why not? (a useful, medical application?)