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Monitoring of pH and metabolites in wound healing processes

Empa
Materials Science and Technology

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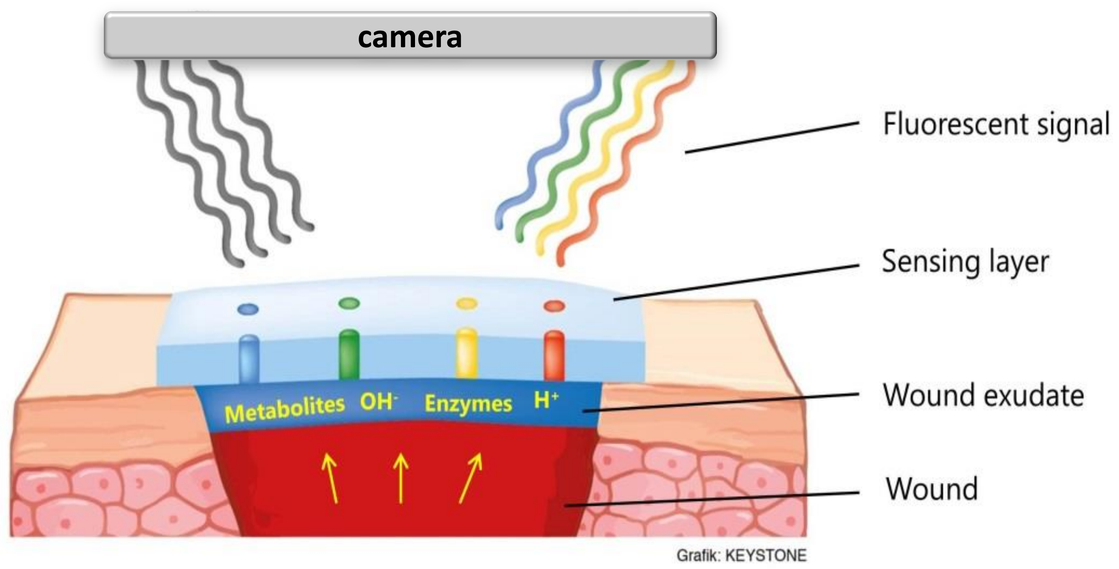
Introduction

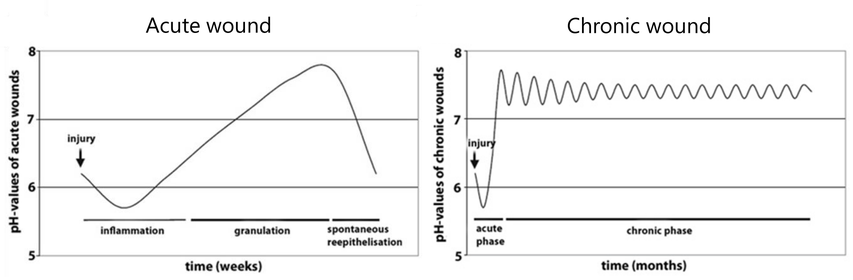
Wound management is a common problem in patients of advanced age. Various factors such as diabetes increase the occurrence of **chronic wounds**, which then require an intensive care and frequent change of wound dressing.

We demonstrate a novel wound pad for **noninvasive monitoring** of the healing phase without the need to remove the dressing, what could damage the newly-grown tissue. The system detects changes in the most relevant wound parameters, pH and glucose concentration, and generates a fluorescent signal measurable with a fluorescence lifetime camera. For monitoring of pH, a fluorescein-derived dye was selected; for glucose quantification, glucose oxidase and horseradish peroxidase were used. Both sensors were immobilized on a biocompatible matrix to develop a functional coating for wound pad application.



pH sensing

pH of the wound fluctuates during healing and the pattern is different for acute and chronic wounds. It allows to distinguish between these two states.



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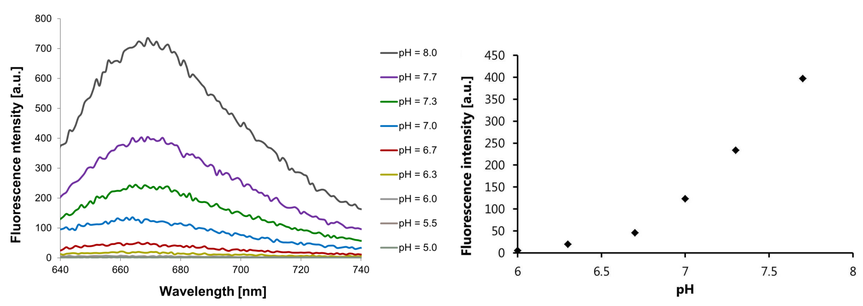
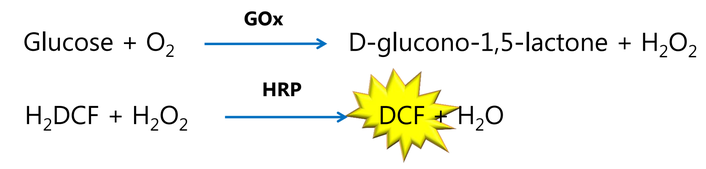
For pH sensing the 5(6)-carboxynaphthofluorescein was used. The dye was immobilized on agarose matrix, and the fluorescence spectra were recorded after incubation in PBS buffer of different pH-values. The fluorescence intensity increases with increasing pH.



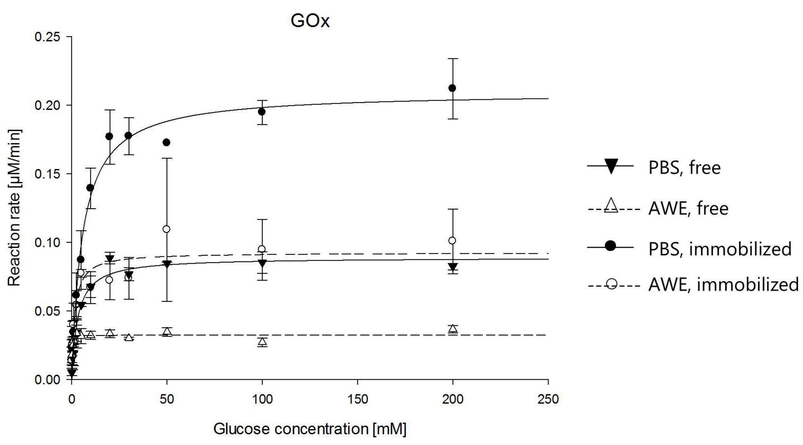
Glucose sensing

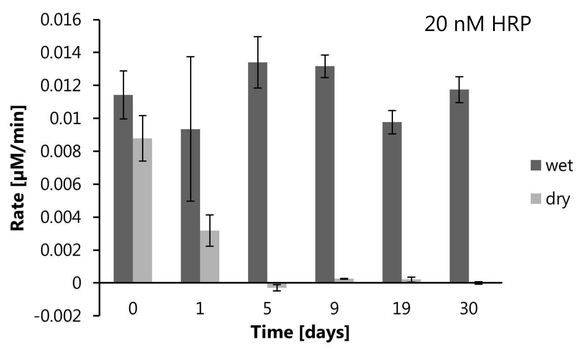
Glucose oxidase (GOx) and horseradish peroxidase (HRP) convert a non-fluorescent substrate into a fluorescent product.

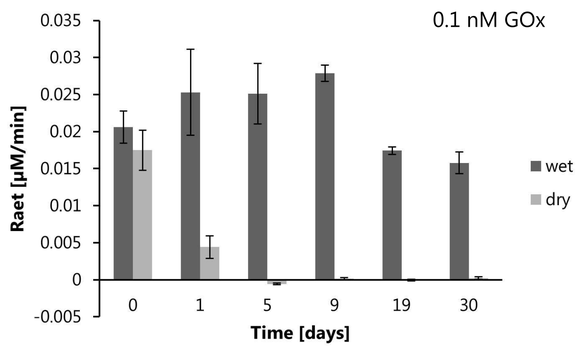


Enzymes were immobilized in alginate. Their catalytic activity was determined in buffer (PBS) and artificial wound exudate (AWE).

Glucose oxidase reaction rate is 2-fold higher after immobilization (●,o) than in a free form (Δ,▼). Ater enzyme bounding to a matrix, its k_{cat} increased almost 2-fold in artificial wound extract.





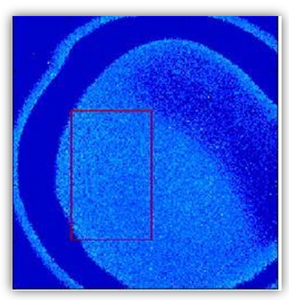


Stability of the functionalized alginate matrix with GOx and HRP. The gels were stored **at 30°C** in moist or dry conditions.

Even after **30 days** the enzymes retained their activity, if kept moist. As soon as the matrix dried, the enzymatic activity was compromised.

Camera detector

A custom-made fluorescence lifetime reader, developed by CSEM, measures the lifetime of the fluorescence signal.



Fluorescence signal of matrix with 0.1 nM GOx, 20 nM HRP and 50 mM glucose.

Camera development:
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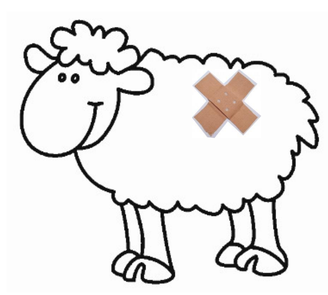
Conclusions and future work

- Enzymes and dyes could be immobilized to fabricate functional wound pads.
- The pH and glucose concentration can be monitored based on fluorescence.
- The glucose sensor system is very stable after immobilization on alginate matrix.
- The glucose sensor can easily be adapted to the detection of other specific metabolites by replacing the GOx with other specific oxidases.
- The monitoring can be performed by a portable fluorescence camera in a noninvasive way.



Work to be submitted as:
Jankowska *et al.* „Simultaneous detection of pH fluctuations and metabolites for wound monitoring applications."

Acknowledgement
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