Optical Environment Sensing
-Multimodal Measurement of Cell Culture Conditions-

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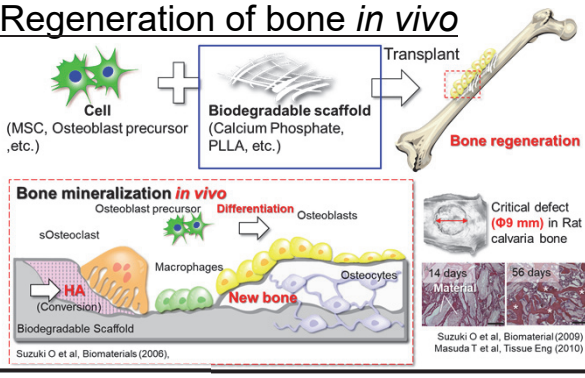
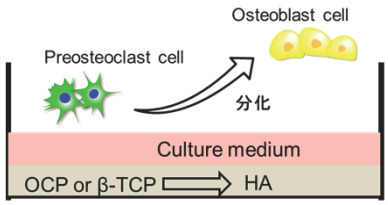
培養面のpH・温度・Ca^{2+}を長時間・安定計測!!

Background

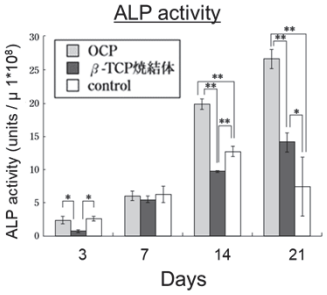
Regeneration of bone *in vivo*



Conventional simulator for catheter ablation



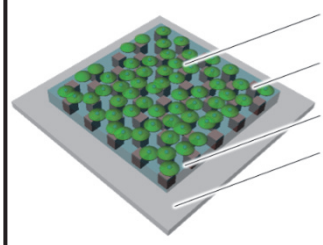
OCP conversion to HA

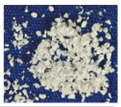


Measurement of culture condition during conversion is important.

Concept

Multimodal measurement of cell culture condition by fluorescence sensors





Octa Calcium Phosphate (OCP)

OCP (OctaCalcium Phosphate) $[Ca_8H_2(PO_4)_6 \cdot 5H_2O]$ → HA (HydoroxyApatite) $[Ca_{10}(PO_4)_6(OH)_2]$

Environmental change during conversion of OCP to HA

O Suzuki et al, Biomaterial (2006)

(1) Hydorolysis

$$5Ca_8H_2(PO_4)_6 \cdot 5H_2O \rightarrow 4Ca_{10}(PO_4)_6(OH)_2 + 6H_3PO_4 + 17H_2O$$

(OCP → HA)

Ionization of H_3PO_4: $H_3PO_4 \rightleftarrows H^+ + H_2PO_4^-$, $H_2PO_4^- \rightleftarrows H^+ + HPO_4^{2-}$, $HPO_4^{2-} \rightleftarrows H^+ + PO_4^{3-}$

(2) $[Ca^{2+}]$ consumption

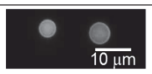
$$Ca_8H_2(PO_4)_6 \cdot 5H_2O + 2Ca^{2+} \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 3H_2O + 4H^+$$

(OCP → HA)

Measurement of pH and Ca^{2+} change during OCP conversion to HA

OCP conversion to HA

Conventional method for physiological sensing

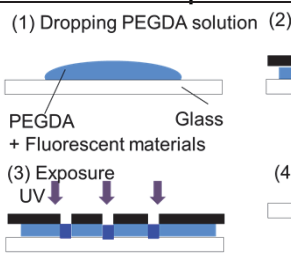
Method	Electrode	Fluorescence
Photo	 O_2 Microsensor (Unisense)	 10 μm H. Maruyama, et.al., Trans. Mech (2011)
Multimodal sensing	Possible	Possible
Spatial resolution	Low	High

Fluorescence dyes for Multimodal sensing

	Dye	Excitation nm	Emission nm
pH	FITC	488	515
Ca^{2+}	Fura-2	360	500
Temp.	Rhodamine B	561	610

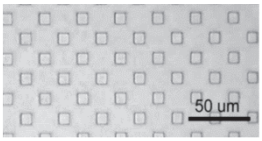
Fabrication of hydrogel sensor

Fabrication process



Materials of sensors

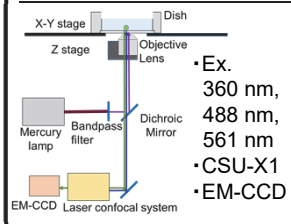
- DI water : 55%
- PEGDA : 30%
- Omnirad 1173 : 5%
- Fura-2+DI water : 5%
- FITC+DMSO : 5%
- Rhodamine b : 1wt%



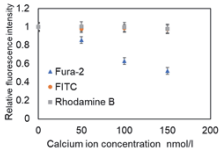
Fabricated fluorescence sensor

Experiment

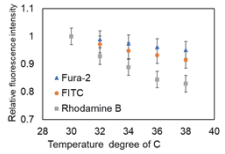
Calibration of fluorescence sensor



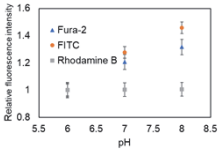
- Ex. 360 nm, 488 nm, 561 nm
- CSU-X1
- EM-CCD



Ca^{2+} calibration

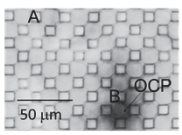


pH calibration

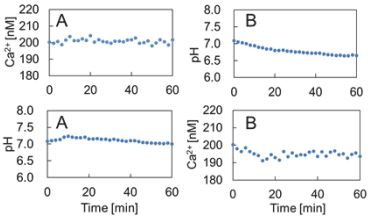


Temp. calibration

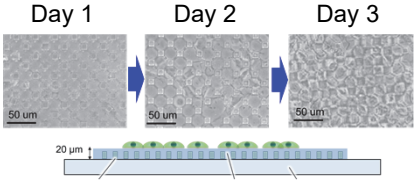
pH and Ca^{2+} sensing by sensor



Initial conditions
pH: 7, Ca^{2+}: 200 nM
Temp. 37 ℃



Cell culture on sensors



Cell: MC3TC-E1

Reference

T. Kobayashi, H. Maruyama, S. Omata, T. Masuda, F. Arai, Optical Environment Sensing -Multimodal Measurement of Cell Culture Conditions- , Robomech2018, 1A1-L04 , 2018

Conclusions

Multimodal sensing of Ca^{2+}, pH during OCP conversion was succeeded.

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