Stimulus responsive micro actuator for biomedical application
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Functional polymeric microstructures are of interest for their potential application to
cmicromachines (microelectromechanical and micro-optomechanical systems), microfluidic systems,
microsensor arrays, and biomedical devices.[1] Polymeric hydrogel materials have been extensively
investigated and can respond to a wide variety of stimuli. Responsive hydrogels are interesting
active materials because they undergo large chain conformational changes upon swelling or under
the influence of external fields. Hydrogel structures can thus retain their responsive properties
when scaled down to the very small dimensions involved in microsystems. Recently, thermally
active microfluidic channels based on a functional hydrogel material were fabricated by Beebe et
al. using lithographic techniques.[2] One key issue with respect to active polymer hydrogels is their
relatively slow response time. It is expected that scaling down hydrogel objects to the micrometer
scale should significantly shorten their response times.

A powerful new approach to the fabrication of polymeric microstructures is two-photon 3D
lithography using two-photon initiated polymerization (TPIP). Two-photon processes have been
used to activate a number of chemical or physical processes and have been utilized in 3D optical
data storage,[3] fluorescence imaging,[4] optical waveguiding,[5] and lithographic microfabrication.[6,7]
The 3D resolution in these processes is facilitated by the unique spatial confinement of excitation
associated with simultaneous absorption of two-photons relative to single-photon mediated
processes. Polymeric microstructures previously fabricated by TPIP have been based on passive
polymeric materials. If microstructures comprised of active polymers can be fabricated by TPIP,
then a new class of stimuli responsive 3D microstructures could be fabricated and investigated for
use in various applications. In this paper, we report the fabrication of a stimulus responsive
hydrogel microstructures by two-photon initiated polymerization (TPIP).

As a first step towards photoactive systems for 3D microfabrication, we have examined
hydrogels containing acetylacetone groups which undergo keto-enol tautomerism and can impart
photoactivity to the system. Hydrogels containing acetylacetone groups were prepared by
polymerization of solutions of acryloylaceton (AA), acrylamide (AAm) and N, N’-methylene
dicaerylamide (MBAAm). [3]-diketone compounds, like acetylacetone, are known to exist in the
form of enol and keto structures which interconvert through a tautomeric equilibrium[8]

Figure 1. Phototautomerization of [3]-diketone compounds

The equilibrium between the keto and enol forms can be shifted under illumination with UV light,
in favor of the keto form. [9] Two-photon polymerization initiators and nonlinear photopolymer
resins have been used to fabricate a wide range of microstructures which are of interest in a number
of emerging technologies. These include micro-optical components, such as tapered wave-guides,
diffraction gratings, and photonic band-gap type structures, as well as simple micromechanical
devices, such as passive cantilever structures. An important parameter that needs to be
characterized and ultimately optimized for a two-photon photopolymer system is the sensitivity. Lower powers and shorter exposure times can be used to fabricate a desired structure as the resin is made more sensitive. The TPiP sensitivity of a range of semi-solid photopolymer resins based on triacrylate monomers have been characterized under exposure with femtosecond pulses at wavelengths in the range of 730 - 800 nm. The threshold power for writing ($P_{th}$) was defined as that power for which a polymeric structure could be fabricated that was adequately cross-linked so as to survive the developing stage. $P_{th}$ values of 0.2 to 0.3 mW were obtained for dye 4 and a related dye as two-photon initiators in the triacrylate based resin. Similar threshold measurements were performed for AA/AAm/MBAAm comonomer solutions with dye 4 again acting as the two-photon initiator. $P_{th}$ was found to be 0.4 mW at 730 nm with for this hydrogel forming co-monomer solution. Optically induced damage was observed for resins with or without 4 at powers greater than 10 mW (damage power, $P_{D}$). The dynamic writing power range for polymerization ($P_{D} / P_{th}$) is thus 25, for the gel forming system and is very comparable to the results for the triacrylate resin. This indicates that the AA, AAm, and/or MBAAm monomers have a quantum yield for radical initiation by dye 4 that is comparable to that for the acrylate monomer, and that they are effective monomers for two-photon resins with this initiator. In the absence of initiator, no polymerization occurred at powers below the $P_{D}$.

Under UV illumination, the degree of swelling of the hydrogel increases with time, but the dimension of the gel did not change under dark conditions after exposure, indicating that the swelling is essentially irreversible. While the mechanism of swelling in the gels is not yet clear, we observed that the pH of water in the presence of gel decreases from 7 to 4 during UV irradiation. It is possible that proton generation in the gel results in swelling due to difference in osmotic pressure between inside and outside of hydrogel.

The microcantilever was anchored on a fused silica substrate by TPiP process. A collimated UV beam for driving actuator was delivered to the underside of the microstructure. The cantilever deflects upwards by ~ 45° after illumination with an intensity of 3 mW/cm² at 244 nm for 20 minutes. This is consistent with a swelling of the bottom of cantilever induced by UV excitation. The 20 min. response time of deflection considerably shorter than the time for photoinduced swelling of the bulk specimens described above. By choosing proper hydrogel and photo initiators, the resolution of micro-cantilever and response time will be drastically improved. A key advantage of such optically powered microactuators is that they can be controlled remotely without an electrical connection. In our system, the response time of cantilever is mainly dominated by kinetics of photochemical reaction rather than diffusion process.

References
Stimulus-responsive Microactuator for Biomedical Application

Toshiyuki Watanabe, Noriko Kimura, Youmei Lu, Fuyuki Hasegawa, Megumi Akiyama, Kenro Totani
Tokyo University of Agri. & Tech.
Iichiro Harada, Toshihiro Akaike
Tokyo Institute of Technology
Outline of this talk

Introduction
Two-photon initiated polymerization
Photochemical reaction during two-photon initiated polymerization
Role of co-initiator
Microfabrication of hydrogel for biomedical application
Applications of Hydrogel

Stimuli responsive
- pH
- Temperature
- Electric fields
- Light
- Carbohydrates (glucose)

Current Applications
- Biosensors (diagnosis of diabetes)
- Micro fluidic system (bulb)
- Micro machine (actuator)

Function of hydrogel was ascribed to volume-phase transition.

Diffusion process is proportional to $L^2/D$

$L$: diameter, $D$: diffusion coefficient

Advantage of sub nanoscale hydrogel
- Quick response
Fabrication limitation of hydrogel

Resolution of Polymerization
  • Photo-mask depth > 30 µm

Time Response
  • Functional structures decrease response time from 30 min. to 10 sec.

Two-Photon Processes Provide 3-D Resolution

One-Photon Excitation

Two-Photon Excitation

Excitation is confined to volume very close to focus where intensity is highest → Pinpoint 3D resolution
Two-Photon Processes Provide Improved Penetration of Light Into Absorbing Materials

Excitation by one photon results in absorption by the surrounding medium before the beam reaches the sample.

Excitation by two photons of half the energy allows for penetration through the material, and then two photons can be absorbed by the sample.
3D microfabrication by two-photon excitation


Two-Photon Excited Processes

\[ S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_n \]

Two-photon Absorptivity \( \delta \)

Electron Transfer
Energy Transfer
Photochemistry

\[ h\nu_A \]

Fluorescence \( \Phi_{fl} \)

I

S. R. Marder et al., Science, 271, 335 (1996)
Objective
Microfabrication of hydrogel by TPIP
Application of TPIP to biomedical device
Scaffold for tissue engineering
Bulb for microfluidic system
What is important factor to control cell functions

**Fabrication**
- Optimization of elastic modulus of ECM
- Nano-patterned ECM

**Measurement**
- Localization, diffusion and fluctuation of protein
- Kinetics and dynamics of filament

- Cytokine receptor
- Transporter/Channel
- Actin filament
- Microtubule fiber
- Intermediate filament
- Surface materials
  - Fibronectin
  - Collagen fibril

**Stimulating factor**
(Elastic modulus of ECM)

**Signal from ECM ligand**

**Precise control of ECM components**

Nonlinear optical processes provide suitable tools for tissue engineering
Project 1

1) Fabrication of elastic nano posts using two-photon initiated polymerization
2) Optimization of substrate composition of hydrogel for tissue engineering
3) Culture cells on elastic micro posts
4) Measurement of force in cell using elastic posts
5) Verification of the mechanochemical and tensegrity model
Polymerization by using BSDMB dye

Components of sample

- **Monomer**: 2-hydroxyethyl acrylate (HEA)
- **Cross-linker**: ethylene diacrylate (EDA)
- **Initiator**: BSDMB
- **Solvent**: 1.4-dioxane

**Components Structures**
- HEA
- EDA
- BSDMB

**Spectra**

The one-photon and two-photon absorption spectra of BSDMB

δ : two-photon absorption cross section

GM = 10^{-50} \text{ cm}^4 \cdot \text{s/photon} \cdot \text{molecule}
Optical microscope image of hydrogel

SEM image of hydrogel
Evaporation or ionization

<table>
<thead>
<tr>
<th>Composition</th>
<th>Elastic modulus /Pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEA:EDA=1:0.10</td>
<td>$2.75 \times 10^4$</td>
</tr>
<tr>
<td>HEA:EDA=1:0.05</td>
<td>$1.2 \times 10^3$</td>
</tr>
</tbody>
</table>
Photophysical process of initiator

\[ \text{Chromophore} \quad \xrightarrow{\text{S}_1} \quad \text{Fluorescence} \quad \phi_f \approx 0.5 \]

\[ \text{S}_0 \quad \xrightarrow{h\nu} \quad \text{S}_1 \quad \xrightarrow{\phi_T \approx 0} \quad \text{T}_1 \quad \xrightarrow{\text{Intersystem crossing}} \quad \phi_{et} \approx 0.5 \]

\[ \text{S}_1 \quad \xrightarrow{\phi_{backet} \approx 1} \quad \text{Monomer} \]

Electron transfer to monomer
**Polymerization by using eosinY**

**Components of sample**

- **Monomer**: 2-hydroxyethyl acrylate (HEA)
- **Cross-linker**: Ethylene diacrylate (EDA)
  
  HEA: EDA = 1:0.1 (molar ratio)
- **Co-initiator**: 0.1M triethanol amine aq
- **Initiator**: eosinY
- **Solvent**: H₂O
- **Polymer binder**: poly(acrylic acid)

The one-photon and two-photon absorption spectra of eosinY

![Graph showing the absorption spectra of eosinY](image)
SEM images of hydrogel posts
<table>
<thead>
<tr>
<th>Composition</th>
<th>Elastic modulus /Pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEA:EDA=1:0.10</td>
<td>2.0x10³</td>
</tr>
</tbody>
</table>

Glass capillary
Synthesis of photo-responsive polymer gel

Initiator: AIBN
Solvent: DMSO
Temperature: 60°C
Time: 22hr.

R = CH₃ or OC₂H₅
Fabrication of micro-machine by two-photon initiated polymerization

Drive of cantilever by UV light based on tautomerization

Stereomicroscope image of cantilever before UV irradiation

Stereomicroscope image of cantilever after UV irradiation 15 min.

Summary

• Ring structure of hydrogel was obtained by illuminating strong laser beam due to thermal effects rather than electron transfer.
• Back electron transfer from radical anion of monomer to initiator prevent two photon initiated polymerization.
• Co-initiator drastically improve rate of polymerization.
• TPIP is promising microfabrication process which offers various biomedical applications.
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