Adding biomolecular recognition capability to 3D printed objects: 4D printing

C.A. Mandon, L.J. Blum and C.A. Marquette

Université Lyon 1 - CNRS 5246 ICBMS
The 3d.FAB platform is an **additive manufacturing platform**, dedicated to academic and private innovation, through **3D printing for health**

**Open source** and application driven **approach**

**Available printers:**

- Polyjet Objet 30 by Stratasys
- FDM (Fused Deposition Modeling) by Tobeca®
- Photo-polymerization with DLS (Digital Light Processing) by B9 Creator

**Technologies**

- Hardened materials for rapid prototyping of diagnostic devices with multicomponent possibilities
- Soft materials for biology: the future of medicine, via bio-extrusion and photo-polymerization with a large range of hydrogel like polymer
Specialized in the **biology/material interactions** and the development of **biosensing systems**, we aim at filling the gap between technology and application using additive technology.

2 main areas:

- **Regenerative medicine** (dedicated living cells and tissues bioprinters)
- **Biochemistry for diagnosis** (prototyping of 3D lab-on-chip, novel materials for 3D medical devices, biocompatible polymers, cell-size 3D printing, **4D**, ...)

![Diagram showing 1D, 2D, 3D, and 4D perspectives with biosensing, catalysis, and biointeraction complex functions.](image-url)
4D printing

1D | Monomer

2D | Polymer

3D | Specific forms

4D | Printing of “smart” biological structures

Enzyme in solution (batch system)

Immobilized enzyme (ELISA Test)

Multiparametric detection (Microarray)

The 4th dimension is represented by an activity. Either physical, electronical, chemical, biological or biochemical.
Polyjet = inkjet + photopolymer

Polyjet Objet 30 (Stratasys)

Printing of functional devices:

Hardened materials for rapid prototyping of diagnostic devices with multicomponent possibilities
FDM: Fused Deposition Modeling

Hot extrusion
Multiple extruders

Rapid prototyping of passive devices (non-functional)

Tools for pre-production prototypes (visualization and validation)
Bioprinting

Cold extrusion

Extruder

Syringe

Bio-ink

Model

Build platform

Tools for producing 3D objects incorporating living elements

Biocompatible hydrogels printing
Cutaneous substitute

Bioprinted dermis

Bioprinted skin engineered with human primary skin cells

(Patent deposit n°15 63461, 12/30/2015)
DLS : Digital Light Processing (B9 Creator)

**SLA: StereoLithography Apparatus**

- High precision: $Z (6.3 \mu m$) et en $X/Y (50 \mu m$ par pixel)
- Resin photopolymerisation - Compatible tools for hydrogel formulations

- ➢ 4D printing tools
Ultra-high-precision down to 140 nm

Production of objects at the living cells' scale
DLS : Digital Light Processing (B9 Creator)

4D printing tools
Our lab is specialized in the development of diagnostic tools
4D: hydrogel formulation

**hydrogel**

\[ \text{H}_2\text{C} = \underbrace{\text{C} = \text{O}}_{\text{O}} \underbrace{\text{C} - \text{O}}_{\text{n}} \text{C} - \text{C} \text{H}_2 \]

Poly(ethylene glycol) diacrylate (PEGDA)

\[ \text{Free radical based initiator} \]

\[ \begin{array}{c}
\text{C} \cdot \\
\text{O} \\
\overbrace{\text{O}}_{\text{P}} \text{P} \\
\text{Cl}
\end{array} \]

\[ + \]

Visible light (420 nm)

**4D hydrogel**

\[ \begin{array}{c}
\text{H}_2\text{C} = \text{CH} \\
\text{C} \cdot \\
\text{O} \\
\underbrace{\text{C} - \underbrace{\text{CH}_2 - \text{O}}_{\text{n}} \text{C} - \text{CH} = \text{CH}_2}
\end{array} \]

\[ + \]

Photoinitiator (Irgacure 819)

Active biomolecule

\[ \begin{array}{c}
\text{h} \\
\nu
\end{array} \]

\[ \text{Visible light (420 nm)} \]
**Immuno test**

ELISA (Sandwich)

**Protocol:**
- Saturation
- Simultaneous incubation: BNP, Anti-BNP-biot, SAV-PA
- Washing chimiluminescent substrat incubation (CdP Star®)

**Hydrogel:**
- Cresol Red
- PEG-700-DA
- Irgacure I819
- +/- Anti-BNP

**Protocol:**
- Saturation
- Simultaneous incubation: BNP, Anti-BNP-biot, SAV-PA
- Washing chimiluminescent substrat incubation (CdP Star®)
Chimiluminescent test

**Protocol:**
Veronal Buffer 30 mM KCl 30 mM pH 8.5
Glc 100 mM
Luminol 220 µM

**Hydrogel:**
Cresol red
PEG-700-DA
Photoinitiator (Irgacure I819)

+ GOX + HRP
simultaneously printed

**GOX**
Glc + H₂O + O₂ → Gluconic acid + H₂O₂

**HRP**
Luminol + H₂O₂ → 3-aminophthalate + hν (425 nm)
4D: Biosensing applications

Chimiluminescent test

Glc + H₂O + O₂  \xrightarrow{GOX}  Gluconic acid + H₂O₂
Luminol + H₂O₂  \xrightarrow{HRP}  3-aminophtalate + hν (425 nm)

Hydrogel:
Cresol red
PEG-700-DA
Photoinitiator (Irgacure I819)
+ GOX + HRP
simultaneously printed

Protocol:
Veronal Buffer 30 mM KCl 30 mM pH 8.5
Glc 100 mM
Luminol 220 µM
4D: Biosensing applications

Chimiluminescent test

Glc + H₂O + O₂ → Gluconic acid + H₂O₂
Luminol + H₂O₂ → 3-aminophtalate + hν (425 nm)

Hydrogel:
Cresol red
PEG-700-DA
Photoinitiator (Irgacure I819)

+ GOX + HRP
Successively added

Protocol:
Veronal Buffer 30 mM KCl 30 mM pH 8.5
Glc 100 mM
Luminol 220 µM
We have validated the use of such complex devices for catalysis, biointeractions and bioprinting (skin, cartilage)

- Other biointeractions: DNA, RNA interaction detection,
- Complex functions: development of systems in motion, microfluidic systems, electronic devices,
Thank you for your attention.