Pulsed laser deposition, laser direct writing, and two photon polymerization of materials for medical devices

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Comparison of rapid prototyping technologies used in medical devices and tissue engineering

<table>
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<tr>
<th>Rapid Prototyping Technique</th>
<th>Resolution (µm)</th>
<th>Strengths</th>
<th>Weaknesses</th>
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<tr>
<td>3D Bioplotter</td>
<td>250</td>
<td>Compatible with many materials; biomolecules may be incorporated</td>
<td>Completed part may exhibit low mechanical strength; smooth surfaces required; low accuracy; slow processing times</td>
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<tr>
<td>3-dimensional printing™</td>
<td>200</td>
<td>Microscale porosity may be introduced; compatible with many materials; water may be used as binder; no support structure needed; rapid processing times</td>
<td>Material must be in powder form; completed part may exhibit low mechanical strength; powdery surface finish; trapped powder may be present in completed part; may required post processing steps</td>
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<tr>
<td>3-D inkjet printer</td>
<td>180</td>
<td>Compatible with many materials; control of external and internal morphology</td>
<td>Multiple steps involved</td>
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<tr>
<td>3D Fiber-deposition technique</td>
<td>250</td>
<td>Input material in pellet form</td>
<td>High temperature; difficult to prepare structures with microscale porosity</td>
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<tr>
<td>Precision extruding deposition (PED)</td>
<td>250</td>
<td>Input material in pellet form</td>
<td>High temperature; difficult to prepare structures with microscale porosity</td>
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<tr>
<td>Precise extrusion manufacturing (PEM)</td>
<td>200-500</td>
<td>Input material in pellet form</td>
<td>High temperature; difficult to prepare structures with microscale porosity</td>
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<td>Pressure assisted microsyringe (PAM)</td>
<td>10-600</td>
<td>Compatible with many materials; biomolecules may be incorporated</td>
<td>Use of solvent required; small nozzle size inhibits incorporation of particles; narrow range of printable viscosities</td>
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<tr>
<td>Rapid prototyping robotic dispensing system (RPBOD)</td>
<td>400-1000</td>
<td>Compatible with many materials; biomolecules may be incorporated</td>
<td>Precise control of material and medium properties; freeze drying required</td>
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<tr>
<td>Robocasting</td>
<td>100-1000</td>
<td>Compatible with many materials</td>
<td>Precise control of ink properties necessary</td>
</tr>
<tr>
<td>Selective laser sintering (SLS)</td>
<td>500</td>
<td>Microporosity induced in the part; compatible with many materials; no support structure needed; rapid processing times</td>
<td>Material must be in powder form; high temperatures are required; powdery surface finish; trapped powder may be present in completed part</td>
</tr>
<tr>
<td>Stereolithography apparatus (SLA)</td>
<td>250</td>
<td>Compatible with many materials; no support structure needed; rapid processing times</td>
<td>Material must be photocrosslinkable and cytocompatible; involves use of ultraviolet light</td>
</tr>
<tr>
<td>TheriForm™</td>
<td>300</td>
<td>Microporosity induced in the part; compatible with many materials; non-organic binders may be used; no support structures needed; rapid processing times</td>
<td>Material must be in powder form; powdery surface finish; trapped powder may be present in completed part</td>
</tr>
</tbody>
</table>

Direct writing of tissue engineered materials

- (a) experimental apparatus used
- (b) schematic diagram of the matrix assisted pulsed laser evaporation-direct write (MAPLE DW) process

(a) Optical micrograph of MAPLE DW hydroxyapatite ribbon containing regions that have been transferred by the laser. (b)-(d) Optical micrographs of hydroxyapatite patterns on borosilicate glass substrates.

- mesoscopic patterns exhibit the dimensions and boundaries found in the ribbon.

a highly porous network with a 0.5 mm-6 mm pore size is observed
pore size and other material parameters are determined by the amount of solvent, the type of solvent, and the size of the ceramic powder

X-ray diffraction pattern

- the MAPLE-DW-transferred material exhibited the composition and phase of the target material
- the X-ray diffraction pattern of MAPLE-DW-transferred hydroxyapatite contains the (200), (111), (002), (102), (210), (211), (112), (300), (202), (310), (222), (320), (213), (004), and (304) peaks of crystalline hydroxyapatite

(a) Optical micrograph and (b) fluorescence image of live-dead stained MG63 osteoblast-like cells 48 hours after MAPLE DW transfer

- the osteoblast-like cells exhibited less well defined boundaries than ceramics due to growth and proliferation
- live-dead assay results indicated that 100% of the osteoblast-like cells remained viable after MAPLE DW transfer

(a) MG 63-hydroxyapatite composite MAPLE DW ribbon containing regions that have been transferred by the laser. 
(b) MAPLE DW transferred MG 63-hydroxyapatite composites.

- the MAPLE DW-transferred osteoblast-like cells exhibit cytoplasmic extensions

(a) Optical micrograph of the cell-ceramic composite taken 72 hours after MAPLE DW transfer
(b) Live dead assay-stained fluorescence micrograph of the cell-ceramic composite taken 72 hours after MAPLE DW transfer

- These micrographs demonstrate osteoblast-like cell growth and proliferation after laser transfer
- Live-dead staining indicates that the transferred cells remain viable

Growth profile of MG63 osteoblast-like cells in EMEM(EBSS) media, MAPLE DW transferred MG63 osteoblast-like cells, and MAPLE DW-transferred MG63 osteoblast-like cell-hydroxyapatite composites.

near simultaneous absorption of two photons creates a so-called virtual state for several femtoseconds

electronic excitation is analogous to electronic excitation by a single photon with a much higher energy

negligible absorption occurs except in the immediate vicinity of the focal volume of the light beam

Two photon polymerization parameters

- dependent on a number of fabrication conditions and system parameters:
  - laser power
  - exposure time
  - distance between two voxels (volumetric pixel)
  - truncation amount of a voxel
  - numerical aperture (NA) of the objective lens
  - sensitivity of photoinitiator
Tissue engineering scaffolds

- two photon induced polymerization was used to develop Lego®-like interlocking tissue engineering scaffolds, which contain arrays of cylindrical pillar structures on both sides of a flat Ormocer® chip
- these structures may be used either as free-standing scaffolds or stackable blocks for layer-by-layer replication of heterogeneous tissues
- schematic and an optical micrograph of a free standing Lego®-like scaffold fabricated by two photon induced polymerization are shown; the pillars are 75 µm in diameter and 20 µm in height

Fluorescent micrographs at several magnifications of B35 neuroblast-like cells on an Ormocer® pillar 48 h after seeding

- the cytoskeleton is denoted by a green color and the nuclei are denoted by a blue color
- cells seeded on these structures are oriented along the pillar walls, and were found to gradually increase in number over time.
- 100% of the B35 neuroblast-like cells remained viable 48 hours after inoculation on the Ormocer® substrates.

Tissue engineering scaffold created out of zirconium oxide hybrid material by means of two-photon polymerization. (a) Scanning electron micrograph of a portion of the tissue engineering scaffold, which contained an array of hollow cylinders (radius=50 µm). (b) Scanning electron micrograph of three hexagonal tissue engineering scaffolds; the topmost scaffold contained four layers, the rightmost scaffold contained three layers, and the bottommost scaffold contained two layers. (c) Micro-computed tomography scan from within one layer of a seven-layer tissue engineering scaffold.

Tissue engineering scaffold created out of zirconium oxide hybrid material by means of an indirect rapid prototyping approach. (a) Scanning electron micrograph of a portion of a replica tissue engineering scaffold, which contained an array of hollow cylinders. (b) Scanning electron micrograph of a replica hexagonal tissue engineering scaffold; this replica scaffold contained three layers.

Microneedles for drug delivery

- flexibility of the two photon polymerization technique allows the size, geometry, and mechanical properties of the microneedle to be readily modified
- for example, the dimensions of a hollow microneedle can be readily modified

Griss et al. have previously shown that out-of-plane microneedles created using a multistep reactive ion etching/passivation process do not suffer from obstructions caused by loose skin.

- out-of-plane hollow microneedles containing flow channels positioned off-center with respect to the needle tip were fabricated using two photon polymerization.
- aspect ratio was varied by altering the diameter of the microneedle base.
- ripple-like features were attributed to the layer-by-layer fabrication approach.
- the length of these microneedle (800 µm) enables possible use for delivery of pharmacologic agents as well as withdrawal of biological fluids.
MTT assay of acrylate-based polymer containing 10-25 % wt urethane dimethacrylate and 10-20% tetrahydrofurfuryl-2-methacrylate

- comprised of 10-25 % wt urethane dimethacrylate (CAS 72869-86-4) and 10-20% tetrahydrofurfuryl-2-methacrylate (CAS 2455-24-5)
- described by manufacturer as “Class-IIa biocompatible”; previously examined under ISO 10993
- polystyrene wells were modified with corona discharge in order to incorporate oxygen-containing chemical groups within surface polystyrene chains; increase surface hydrophilicity; and enhance cell spreading and attachment
- the acrylate-based polymer did not exhibit cytotoxicity in a manner that would raise concerns regarding potential in vivo use in a microneedle device

Two photon polymerization of acrylate polymer microneedle array on glass slide

- scanning electron microscopy images obtained at 45° tilt of e-shell 300 hollow microneedles on glass substrates, which were produced using two photon polymerization.
  - a) Image of 614 +/- 12 μm long microneedle array.
  - b) Image of 710 +/- 10 μm long microneedle array.
  - c) Image of 710 +/- 10 μm long individual microneedle.
  - d) Image of 710 +/- 10 μm long individual microneedle
- the base diameter of these microneedles is 226 +/- 5 μm
- dimensions are shown as average +/- standard deviation

Freestanding microneedle arrays—microneedles and substrate

- the 5x objective was used instead of the 10x objective due to limitations associated with radial laser intensity degradation in the focal plane
- in moving from the 10x objective to the 5x objective, the radial laser energy degradation was reduced
- changing the objective resulted in a reduction in two photon processing resolution
Delivery of quantum dots (QDs)

- 2-10 nm diameter fluorescent semiconductor nanoparticles
- Bandgap values for quantum dots increase as the nanoparticle radius decreases
- Passive mechanisms -> preferential retention of quantum dots within tumor cells
- Active mechanisms -> quantum dots may also be conjugated with peptides, antibodies, aptamers, pharmacologic agents, and other tumor-specific molecules

Two photon polymerization of acrylate polymer microneedle array on acrylate polymer substrate

(Left) diagram of hollow microneedle device fabrication by means of two photon polymerization. (Right) scanning electron microscopy images of e-shell 300 hollow microneedle array, which was produced using two photon polymerization

a) image of microneedle array obtained at 45° tilt
b) image of individual microneedle obtained at 45° tilt
c) image of individual microneedle obtained at 0° tilt

The length and base diameters of these microneedles are 508 +/- 33 µm and 212 +/- 3 µm, respectively.

Multiphoton microscopy images of quantum dot injection via two photon polymerization-fabricated e-shell 300 microneedle array as well as via topical application

- The microneedles are presented as surface renderings (in gray) and the quantum dots are presented as maximum projections (in red)
- (a) Microneedle in porcine skin prior to quantum dot injection
- (b) Microneedle in porcine skin after quantum dot injection
- A broad distribution of the quantum dots in the deep epidermis and dermis was observed within fifteen minutes
- (c) Quantum dots topically applied to porcine skin
- The topically applied quantum dots exhibited poor penetration and remained in the topmost 50 μm region of the epidermis
- Levene et al. recently demonstrated the use of gradient index lenses for imaging of deeper tissues

Discussion

- Two photon polymerization is a novel technique for processing hybrid organic-inorganic materials and acrylate-based polymers.
- Our results suggest that two photon polymerization is able to create small-scale devices with a larger range of geometries than conventional microfabrication and nanofabrication techniques.
- Work is underway to expand the type and number of organic and inorganic building blocks (novel organic $\pi$-conjugated chromophores) that are compatible with continuous wave laser or nanosecond pulsed laser processing.
- Two photon polymerization of materials containing biologically active molecules and biodegradable materials will also be of benefit for medical device applications.