Laser Zentrum Hannover, Germany

3D Nanotechnology and Laser Printing of Nanoparticles and Living Cells

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High resolution manufacturing with lasers

Two-photon polymerization  Laser printing  Laser ablation
Micro- and nanostructuring for better implant adaptation

Implant → Interaction with the tissue consisting of different cell types

- "bad" cells → Isolation and loss of functions
- "good" cells → Reconstruction and regeneration

- fibroblasts
- neuronal cells, osteoblasts, endothelial cells, chondrocytes, etc.
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Material Functionalization

Biology

- Growth Factors
- Adhesion Peptides

Chemistry

- In general
- Wettability

Physics

- Elasticity
- Topography
- Surface Charge

Selective cell control and antibacterial effect
Laser microstructuring of metals: Titanium “lotus-like” structures

Combination of micro- and nanostructures
Impact on Fibroblasts: Morphology

Schlie S et al. (2010), Fadeeva E et al. (2010a), Fadeeva E et al. (2010b), Schlie S et al. (2012)

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Primary neurons on laser-generated spike structures

Staining of neuronal markers and nucleus (blue)

Beta III Tubulin (green)  MAP 2 (red) and synaptophysin (green)

Results:
Spike structures do not have a negative effect on primary neurons
Laser generation of nanoparticles

- High purity and stability
- Monoatomic materials
- Alloy nanoparticles
- Particle surface-functionalization
- Polymer-embedded nanoparticle
- Coatings with nanosized particles
- Controlled drug-release
- Stoichiometric nanoparticles
- Novel methods → better control

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Laser printing of spherical gold and silicon nanoparticles

Jet and droplet formation

\[ E_p = 65\text{nJ} \]

\[ E_p = 70\text{nJ} \]

\[ E_p = 75\text{nJ} \]

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Fabrication of spherical nanoparticles by laser printing

Receiver substrate (glass)

Thin Au film

Donor substrate (glass)

Tightly focused fs laser pulse

“Laser-induced transfer of metallic nanodroplets for plasmonics and metamaterial application“ JOSA B, Vol. 26, No. 12, B130, 2009
"Nano"-letters from Au

- SEM-image of nanoparticles

- The same image made by darkfield microscopy
Laser induced transfer of silicon nanoparticles from bulk silicon

Magnetic response of Si spherical nanoparticles (Mie theory)

Extinction of spherical Si nanoparticles in air

\[ \sigma_{\text{ext}} = \sum_{l=1}^{\infty} \sigma_{l}^{E} + \sigma_{l}^{M} \]

Extinction cross section

Magnetic dipole (md)
Electric dipole (ed)

Resonant response of silicon nanoparticles

Controlled fabrication and precise deposition of silicon nanoparticles

Laser induced crystallization

 Receiver substrate (glass)  Square-shaped fs laser pulse

 Silicon nanoparticle (c-Si)  Silicon nanoparticle (a-Si)

Selective phase change of Si nanoparticles

Nonlinear interaction of fs-pulses

Linear excitation

Non-linear excitation

E_0 \rightarrow s_0

1P induced Fluorescence

E_1 \rightarrow s_1

E_0 \rightarrow s_0

2P induced Fluorescence

Fs-laser pulses allow energy deposition into a Volume!
Two-photon polymerization
3D nanostructuring by two-photon polymerization


Deutsches Patent 101 52 878.7-43
Two-photon polymerization (5cm/s)

Schematic representation of the experimental setup

Commercially available 2PP system from LZH: b.chichkov@lzh.de
Regenerative medicine and tissue engineering

Interdisciplinary Research
(Mathematics, Physics, Material Science, Engineering, Biology, Medicine)

Regenerative Medicine:
Replacement or repair of ill organs, which body cannot restore itself

Tissue Engineering:
Fabrication of living tissue from patient cells
Transplantation inside a damaged Organ
Organ transplantation demand

[Image of a cartoon showing a long line of people outside a closed and out of stock heart transplant center.]
Basic idea of tissue engineering

Materials

- Ormocers®
- PEG-DA
- Organic-Inorganic Zr-hybrid materials
- PCL
- PLA
- Gelatin
- E-Shell®

Fabrication of scaffolds via two-photon polymerization (2PP)
Scaffold-based approach / Examples

Fibrin scaffold
3D conductive polymer scaffolds

- PEG-DA and EDOT blends are used for 2PP and sequential *in-situ* oxidative polymerization;
- Real-3D, physically stable and biocompatible microstructures are produced;
- Interpenetrating polymer network of PEG-DA and PEDOT leads to conductivities of up to 0.04 S/cm.

**Opt. Express, 21, 31029 (2013)**
High-aspect 3D two-photon polymerization structuring with widened objective working range (WOW-2PP)

Kotaro Obata, Ayman El-Tamer, Lothar Koch, Ulf Hinze and Boris N Chichkov

We developed a novel two-photon polymerization (2PP) configuration for fabrication of high-aspect three-dimensional (3D) structures, with an overall height larger than working distance of the microscope objective used for laser beam focusing into a photosensitive material. This method is based on a modified optical 2PP setup, where a microscope objective (100× high N.A.), immersion oil and cover glass can be moved together into the photosensitive material, resulting in an effective higher and wider objective working range (WOW-2PP). The proposed technique enables the fabrication of high-aspect structures with sub-micrometer process resolution. 3D structures with a height of 7 mm are demonstrated, which could hardly be built with the conventional 2PP set-up due to refractive index mismatch and laser beam disturbances.

*Light: Science & Applications (2013) 2, e116; doi:10.1038/lsa.2013.72; published online 6 December 2013*

**Keywords:** femtosecond laser; large-scale structuring; laser material processing; photochemistry; two-photon polymerization
Research Article

Two-photon polymerization of polyethylene glycol diacrylate scaffolds with riboflavin and triethanolamine used as a water-soluble photoinitiator

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Aim: In this study, the suitability of a mixture containing riboflavin (vitamin B2) and triethanolamine (TEOHA) as a novel biocompatible photoinitiator for two-photon polymerization (2PP) processing was investigated. Materials & methods: Polyethylene glycol diacrylate was crosslinked using Irgacure® 369, Irgacure 2959 or a riboflavin-TEOHA mixture; biocompatibility of the photopolymer extract solutions was subsequently assessed via endothelial cell proliferation assay, endothelial cell viability assay and single-cell gel electrophoresis (comet) assay. Use of a riboflavin-TEOHA mixture as a photoinitiator for 2PP processing of a tissue engineering scaffold and subsequent seeding of this scaffold with GM-7373 bovine aortic endothelial cells was also demonstrated. Results: The riboflavin-TEOHA mixture was found to produce much more biocompatible scaffolds than those produced with Irgacure 369 or Irgacure 2959. Conclusion: The results suggest that riboflavin is a promising component of photoinitiators for 2PP fabrication of tissue engineering scaffolds and other medically relevant structures (e.g., biomicroelectromechanical systems).
Two-photon polymerization is a technique that involves simultaneous absorption of two photons from a femtosecond laser for selective polymerization of a photosensitive material. In this study, two-photon polymerization was used for layer-by-layer fabrication of 3-D scaffolds composed of an inorganic–organic zirconium oxide hybrid material. Four types of scaffold microarchitectures were created, which exhibit layers of parallel line features at various orientations as well as pores between the line features. Long-term cell culture studies involving human bone marrow stromal cells were conducted using these 3-D scaffolds. Cellular adhesion and proliferation were demonstrated on all of the scaffold types; tissue-like structure was shown to span the pores. This study indicates that two-photon polymerization may be used to create microstructured scaffolds out of an inorganic–organic zirconium oxide hybrid material for use in 3-D tissue culture systems.
Two-photon polymerization/micromolding of microscale barbs for medical applications

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Tissue barbs are small-scale structures that may be used for sutureless joining of tissues. In this study, several types of tissue barbs were fabricated using two-photon polymerization/micromolding, including two-pronged tissue barbs, eight-pronged tissue barbs, 10-pronged tissue barbs, and 16-pronged tissue barbs. Tissue barb penetration in porcine tissue was observed using confocal laser scanning microscopy. Constructs containing medical tape and tissue barbs were created by applying tissue barbs in a parallel arrangement to Transpor\textsuperscript{TM} medical tape. These results suggest that two-photon polymerization/micromolding is an indirect rapid prototyping approach that may be used for high-throughput replication of tissue barbs and other microstructured solid wound sealants.

Keywords: tissue barb; wound sealant; two-photon polymerization; micromolding; indirect rapid prototyping
Hyaluronic Acid Based Materials for Scaffolding via Two-Photon Polymerization

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ABSTRACT: Hydrogels are able to mimic the basic three-dimensional (3D) biological, chemical, and mechanical properties of native tissues. Since hyaluronic acid (HA) is a chief component of human extracellular matrix (ECM), it represents an extremely attractive starting material for the fabrication of scaffolds for tissue engineering. Due to poor mechanical properties of hydrogels, structure fabrication of this material class remains a major challenge. Two-photon polymerization (2PP) is a promising technique for biomedical applications, which allows the fabrication of complex 3D microstructures by moving the laser focus in the volume of a photosensitive material. Chemical modification of hyaluronan allows application of the 2PP technique to this natural material and, thus, precise fabrication of 3D hydrogel constructs. To create materials with tailor-made mechanochemical properties, HA was combined and covalently cross-linked with poly(ethylene glycol) diacrylate (PEGDA) in situ. 2PP was applied for the fabrication of well elaborated 3D HA and HA–PEGDA microstructures. For enhanced biological adaption, HA was functionalized with human epidermal growth factor.
Biological laser printing

LIFT: Laser Induced Forward Transfer
Motivation

Applications in Regenerative Medizin:
- prototyping of complex biomedical products
- biofabrication of tissue substitutes and living organs

Bioprinting  Nozzle free laser printing
LIFT dynamics
EFFECT OF LASER PRINTING ON CELLS – SURVIVAL RATE

Survival rate:

- Fibroblasts (NIH3T3) 98% ± 1%
- Keratinocytes (HaCaT) 98% ± 1%
- Human adipose-derived stem cells (ASC) 99% ± 1%
- Cord blood derived endothelial colony forming cells (ECFC) 98% ± 3%

Nearly all cells survive the printing process.

Live/Dead-staining with Calcein AM (green; vital cells) and Ethidium Homodimer 1 (red; dead cells)

Advantages of Laser assisted Bioprinting:

1. Printing of single to dozen of cells with a micrometer precision
2. No observable damage to the phenotype and genotype of the cells
3. Utilization of cross linkable hydrogels (e.g. Fibrin) enables 3D free form fabrication

Examined cells
- hBMSCs
- hASCs
- ECFCs
- Cardiomyocytes
- Fibroblasts
- Keratinocytes
- Chondrocytes

Assessments
- Survival rates
- Proliferation
- Apoptosis
- Comet assay
- RT-PCR
- Immunohistochemistry

Laser printing has no influence on the cell behaviour

Printed droplets containing endothelial cells (EC, red) or adipose derived stem cells (ASC, green)

After 5 days cultured in VEGF-free media

A printed predefined pattern of cell containing droplets allows to study cell-cell or cell-environment interactions

GENERATION OF 3D SKIN TISSUE

Layers of fibroblasts (NIH3T3) and keratinocytes (HaCaT), in collagen I on Matriderm™

Layered arrangement of red and green HaCaT (eGFP, mCherry), 18 h after printing.

Each color layer consists of four printed sub-layer. The whole construct is about 2 mm high.

The cells have been embedded in collagen directly before printing. The layers do not intermix during or after printing.

Together with Prof. Vogt, MHH
**Generation of skin equivalents by laser printing**

- **Native skin**
- **Matriderm with cells**
- **Matriderm without cells**

Immunofluorescence staining of cytokeratin 14 (green) for keratinocytes cell nuclei were stained with Hoechst 33342 (blue)

Together with Prof. Vogt, MHH
Printed human endothelial cells (green) and human mesenchymal stem cells (hMSC, red) on a cardiac patch

Vessel formation in the printed structure after 8 days (HUVEC/hMSC-co-culture on Matrigel-coated cardiac patch)

Human cells, integrated in the murine vascular network at the border of the cardiac infarct zone, 8 weeks post-infarct

Accelerated vessel formation in printed structure (EC/MSC co-culture) in vitro

Improved heart function after myocard infarction and implantation of printed cardiac patch

Gaebel et al., Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration, Biomaterials 32: 9218-9230 (2011)

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