Poly(vinyl alcohol) based monomers for lithography-based 3D fabrication

C. Heller^{1,2}, M. Schwentenwein², M. Porodec³, M. Schulz-Siegmund³, J. Stampfl¹, F. Varga⁴, R. Liska²

¹ TU Wien, Inst. of Materials Science and Technology, Vienna, Austria

² TU Wien, Inst. of Applied Synthetic Chemistry, Vienna, Austria

³ University of Leipzig, Institute of Pharmacy, Leipzig, Germany

⁴ Ludwig Boltzmann Institute of Osteology, Vienna, Austria

Abstract—Lithography based Solid Freeform Fabrication (SFF) methods (e.g. stereolithography) allow the fabrication of cellular structures with defined pore sizes and geometries. Achievable wall thicknesses range down to 50 µm.

Nevertheless, residual, unreacted acrylate groups have shown to be highly cytotoxic toward osteoblast-like cells and a high local concentration of poly(acrylic acid), formed by in vivo degradation, may impair cell function. Therefore, vinyl ester, vinyl carbonate and vinyl carbamate based monomers, forming harmless poly(vinyl alcohol) upon in-vivo degradation, have been synthesized. They exhibit high photoreactivity and good mechanical properties. In vitro biocompatibility assays revealed that the concentrations leading to cytotoxic cell damage are about two orders of magnitude higher compared to similar acrylate systems.

Keywords—biomaterials, vinyl esters, microstereolithography, surface modification

INTRODUCTION

Solid Freeform Fabrication (SFF) techniques like microstereolithography (µ-SLA) allow the fabrication of structures with defined internal and external geometry (Fig. 1) and have become very appealing methods for the production of three-dimensional scaffolds for several tissue engineering applications in the recent past.[1],[2]

Fig. 1 Concept for SFF based scaffold design

It is based on a layer-by-layer curing of a light-sensitive - most commonly (meth)acrylate based - resin via photopolymerization induced by an UV-Laser. However, the main disadvantage of methacrylates is their limited reactivity and acrylates show a significant tendency towards Michael addition side reactions with proteins or DNA. This frequently results in some significant skin irritancy or sometimes toxic effects making them very controversial for biomedical applications. Further, poly (meth)acrylic acid, formed after hydrolysis, may impair proper cell function.

Fig 2 New monomers

Hence, there is a great need for other polymerizable groups that can be photopolymerized and structured with stereolithography. Therefore, new vinyl esters, carbonates and carbamates (Fig. 2) have been synthesized, giving poly(vinyl alcohol), a water-soluble and biocompatible polymer that has been used for long-term implants upon degradation.[3]

PHOTOREACTIVITY

Photo-Differential Scanning Calorimetry (Photo-DSC) is a unique technique to investigate the polymerization kinetics and the conversion of the double bonds as a function of exposure time and can be used as a benchmark tool considering the suitability of monomers for the µ-SLA process. Samples were weighed into an aluminum DSC pan, purged

with a N₂ and irradiated with filtered UV-light (320-500 nm) from a light guide (8.8 mW/cm² at the surface of the sample). The measurements were carried out with 0.5 wt% of bis(2,4,6-trimethylbenzoyl)-phenylphosphine oxide (Irgacure[®] 819, Ciba[®]) as photoinitiator at ambient temperature. From the heat flow versus time curve characteristic values such as the rate of polymerization (R_p) can be calculated.[4]

BIOCOMPATIBILITY

A. Cell culture

MC3T3-E 1 cells (donated by Dr. Kumegawa, Meikai University, Department of Oral Anatomy, Sakado, Saitama, 35002 Japan) were cultured in alpha MEM (Biochrom, Austria) supplemented with 4.5 g/L glucose, 5% fetal calf serum (FCS, Biochrom, Austria), and 10 µg/mL gentamycin. The cells were kept in humidified air under 5% CO₂ at 37°C. They were subcultured twice a week using 0.001% pronase E and 0.02% EDTA in phosphate-buffered saline (PBS).

B. For cytotoxicity

For the experiments, the cells were seeded at a density of 20,000 cm⁻² in 24-well culture plates and cultured over night. On the next day, the medium was changed and the cells were treated with decreasing concentrations of the monomers for 5 days and compared to untreated cells. Thereafter, cell viability was addressed by incubation of the cultures with a colorimetric growth indicator based on the detection of cellular metabolic activity (EZ4U, Biomedica, Austria). Furthermore, amount of DNA of the cultures were measured by fluorescence with Hoechst 33258 dye as a surrogate of the cell number. Alkaline phosphatase activity was measured in the same cultures by means of p-nitrophenyl-phosphate and normalized to the DNA-amount

C. Cell culture tests

Resin-coated coverslips were placed into a multiwell plate fixed with agarose and sterilized using UV light. MC3T3-E1 cells were seeded at a density of 50,000 cm⁻² onto the and cultured for three days. After the culture time the metabolic activity of living cells was determined by an EZ4U test (Biomedica, Vienna) via photometric measurements (405 vs. 490 nm) and compared to normal cultures (Fig. 3).

Fig. 3 Cells on polymer surfaces

MECHANICAL PROPERTIES

Cylindrical specimens of the polymers were tested using nanoindentation, a fast and material saving method to screen mechanical properties. Samples were indented recording a load versus displacement data indentation hardness (H_{IT}) and indentation modulus (E_{IT}) can be extracted.

MICROSTEREOLITHOGRAPHY

The fabrication of the 3D-parts was done using a µ-SLA system developed by the Laser Zentrum Hannover (LZH), as described previously.[5],[6] A Nd:YAG laser with a frequency multiplied wavelength of 355 nm equipped with an acousto-optic modulator and a scanner was used. The resin used contained Irgacure[®] 819 as PI and 2,2'-dihydroxy-4,4'-dimethoxybenzophenone (HMB) as an UV absorber.

The CAD model of the target structure was sliced into layers of 50 µm. The non-cured resin within the pores of the structure was rinsed with 2-propanol by using an ultrasonic bath before a post curing step under the UV lamp was performed. The resulting structure was examined by means of SEM.

SURFACE MODIFICATION

In order to enable surface modification via covalent protein attachment, resins that have shown promising results as potential scaffold material (vinyl ester) were made amino-reactive by incorporation of cyclic carbonate moieties that have well documented affinity towards free amine groups-VEC (Vinylene carbonate) und PCVC (4-(Hydroxymethyl)-1,3-dioxolan-2-one vinyl carbonate) (7,8,9). Alkaline phosphatase (ALP) was used as a model protein, because of its enzymatic properties, which enable quantitative information on the amount of bound protein, as

well as determination of enzyme bioactivity. With regard to the ethylene carbonate or propylene carbonate functionalities, TRIS buffer (pH 8.5) has been recommended in the literature (7).

Resin-coated coverslips were glued onto well plate surface using neutral curing silicone (Elastosil® 2010). Discs were covered with ALP solution (50 µg ALP/1.5 ml buffer) and incubated for 24 h at 25 °C without stirring. Afterwards, the ALP solution was discarded and discs were thoroughly rinsed with buffer containing 1% SDS and additionally with plain buffer to remove remaining SDS. Discs were then covered with p-nitrophenolphosphate (pNPP) solution and incubated for 30 min at 37 °C. In the presence of ALP, pNPP is enzymatically hydrolyzed into the yellow dye p-nitrophenol. Reaction was stopped using 3M NaOH, the absorbance of the supernatant was quantified at 405 nm using a plate reader and the quantity of adsorbed/covalently bound ALP on the surface was calculated. Amounts as low as 2 ng could be determined using this method.

Significant ($p < 0.01$) covalent immobilization of ALP was found on AVE/VEC and AVE/PCVC surfaces (Figure 5). This suggests that VEC and PCVC could be incorporated into the photo-crosslinked discs in a way that the reactivity was preserved and a covalent attachment of the model protein ALP in its bioactive state was possible.

Figure 4. Cyclic carbonates

Figure 5. Immobilization of ALP on modified surfaces

RESULTS

The results in Table 1 show that vinyl esters, carbonates and carbamates show photoreactivity between those of acry-

lates and methacrylates, comparable mechanical properties and significantly lower cytotoxicity.

Table 1 Properties of new monomers

Monomer	Rp [M L ⁻¹ s ⁻¹]	Cytotoxicity (LD ₅₀) [mM]	indentation modulus [MPa]
Vinyl ester	1.06	~ 5	2290
Vinyl carbonate	0.93	> 10	3910
Vinyl carbamate	0.52	> 10	4340
Acrylate	1.36	~ 0.01	2260
Methacrylate	0.43	~ 1	3170

Considering their overall properties, a vinyl ester based resin was chosen as a suitable scaffold material and could be structured successfully by the means of µ-SLA (Fig. 4).

Fig. 4 Porous structure containing vinyl esters by µ-SLA

CONCLUSION

Vinyl esters, carbonates and carbamates have shown promising results concerning their photoreactivity and mechanical properties in comparison to the established (meth)acrylate references. Further, the biocompatibility, expressed by cell culture tests on polymer specimens and very low cytotoxicity of residual monomers, was determined and suggest making these monomers a suitable starting material for the scaffold production by lithographic techniques. Surface modification of vinyl esters is also possible by incorporation of cyclic carbonates

ACKNOWLEDGMENT

We gratefully acknowledge the financial support by the FWF (P19387)

REFERENCES

1. Lee J, Cuddihy MJ, Kotov NA. (2008) Three-Dimensional Cell Culture Matrices: State of the Art. *Tissue Engineering, Part B: Reviews* 14(1):61-86.
2. Leong KF, Cheah CM, Chua CK. (2003) Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. *Biomaterials* 24(13):2363-2378.
3. Mallapragada SK; McCarthy-Schroeder S. (2000) *Handbook of Pharmaceutical Controlled Release Technology*, Marcel Dekker, 31-46
4. Ullrich G, Ganster B, Salz U, Moszner N, Liska R (2006) Photoinitiators with functional groups. IX. Hydrophilic bisacylphosphine oxides for acidic aqueous formulations. *Journal of Polymer Science, Part A: Polymer Chemistry* 44(5):1686-1700.
5. Baudis S, Heller C, Liska R, Stampfl J, Bergmeister H, Weigel G. (2009) (Meth)acrylate-based photoelastomers as tailored biomaterials for artificial vascular grafts. *Journal of Polymer Science, Part A: Polymer Chemistry* 47(10):2664-2676.
6. Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, et al. (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolithography. *Journal of Micro-mechanics and Microengineering* 18(12):125014/125011-125014/125019.
7. G. Chen, L.van der Does, A. Bantjes, *Journal of Applied Polymer Science* 47, 25□36 (1993)
8. A.L. Nguyen, J. H. T. Luong, *Biotechnology and Bioengineering*, 34, 1186□1190 (1989)
9. Dean C. Webster, Allen L. Crain, *Progress in Organic Coatings*, 40, 275□282 (2000)

Corresponding author: Juergen Stampfl

Institute: TU Wien, Inst. of Materials Science and Technology

Street: Favoritenstraße 9-11

City: Vienna

Country: Austria

Email: jstampfl@pop.tuwien.ac.at