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Biocompatibility of photopolymers for additive manufacturing

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Abstract: To establish photopolymers for the production of class II or class III medical products by additive manufacturing it is essential to know which components of photopolymeric systems, consisting of monomers, photoinitiators and additives, are the determining factors on their biocompatible properties. In this study the leachable substances of a cured photopolymeric system were eluted and identified by HPLC-MS detection. In addition the cured photopolymer was testes for cytotoxicity and genotoxicity according to DIN EN ISO 10993 for long time applications. The results showed that uncured residual monomers are the determining factor on the biocompatible properties of the photopolymeric system. Strategies to reduce these residual monomers in the cured photopolymer are presented.

Keywords: additive manufacturing; biocompatibility; digital light processing; monomers; patient individual medical products; photopolymeric system.

1 Introduction

Additive manufacturing (AM) allows the production of three-dimensional (3D) objects layer by layer, based on a digital model [1, 2]. These models can be designed by using a CAD software or 3d imaging data (e.g. CT, MRI or three dimensional X-ray diagnostics) [3]. Regarding medical applications AM offers the great option to produce patient individual medical devices [4].

Until now the production of class II or class III medical devices by AM has mostly been realized by manufacturing metal alloy implants. However, since also polymers are widely used nowadays for class II or class III medical devices, there is a need to establish polymeric based AM methods for these applications.

A promising polymeric AM method is digital light processing (DLP) which is based on the selective exposer of a photopolymeric system. DLP is especially characterized by high speed, good resolution, and high surface quality [5]. However, so far the use of this technology for long time medical applications is not established yet because there have been very few studies on the biocompatible properties of the photopolymeric systems. Zhu et al. showed in their study that many photopolymers (Watershed 11122XC, Dreve Fototec 7150 Clear, VisiJet SL Clear and Form 1 Clear resin) are not biocompatible as they appear to leach toxic substances [6, 7].

Photopolymeric systems basically consists of three components [8]:

- **Monomers**: long-chain molecules, which ensure the required mechanical properties
- **Photoinitiators**: molecules, which split into radicals after energy input and thus induce the curing reaction
- **Additives**: e.g. UV-stabilisators to prevent uncontrolled curing reactions

To establish photopolymers for production of class II or class III medical products the biocompatibility of the material must be ensured. In this study a highly promising photopolymeric system is evaluated regarding its biocompatible properties.

2 Material and methods

2.1 Additive manufacturing of specimens

According to DIN EN ISO 10993 specimens (50 mm × 10 mm × 1 mm) for the creation of eluates for HPLC-

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MS detection, for cytotoxicity tests and for genotoxicity tests were manufactured (layer thickness 100 µm) using a DLP-printer (FAB-12, pro3dure medical GmbH) and GR-10 (pro3dure medical GmbH) as a photopolymeric system. After the printing process the specimens were cleaned with a 70% isopropanol solution in an ultrasonic bath for 15 min and hardened in an UV-light-chamber for 7 min.

2.2 HPLC-MS detection

To detect leachable substances from the cured specimens, three specimens were stored each in 50 ml water at 37°C for 7 days. As a reference, pure non-cured photopolymer monomers, photoinitiators and UV-stabilisators were dissolved each in deionized water. The extract and the reference solutions were analysed by HPLC-MS (Agilent Technologies Deutschland GmbH). A chromatography column type C-18 with the dimensions 3 µm × 2.1 mm × 125 mm was used.

2.3 Cytotoxicity testing

Cytotoxicity tests were performed with a cell line of fibroblasts (Hs27) and a Cell Counting Kit-8 (Dojindo, CCK-8) according to DIN EN ISO 10993-5 (Tests for *in vitro* cytotoxicity). DMEM was used as extraction medium. The extraction time was 3, 7, 14 and 30 days. According to the norm a reduction of the viability of cells by 30% is considered as a cytotoxic effect.

2.4 Genotoxicity testing

To investigate the genotoxicity, respectively the mutagenicity, a *Salmonella typhimurium* reverse mutation test (Ames test) with extracts of the cured photopolymer was performed according to DIN EN ISO 10993-3 (Tests for genotoxicity, carcinogenicity and reproductive toxicity). The extraction medium was DMSO and the extraction times were 3, 7, 14, and 30 days. The used bacteria strain was *Salmonella typhimurium* TA 18 (Tinova Biochem GmbH). 2-nitrofluorene (2NF) was used as a positive control.

3 Results

3.1 Leachable substances

The upper part of Figure 1 shows the mass spectrum of the extracted solution. In the lower part the exact mass

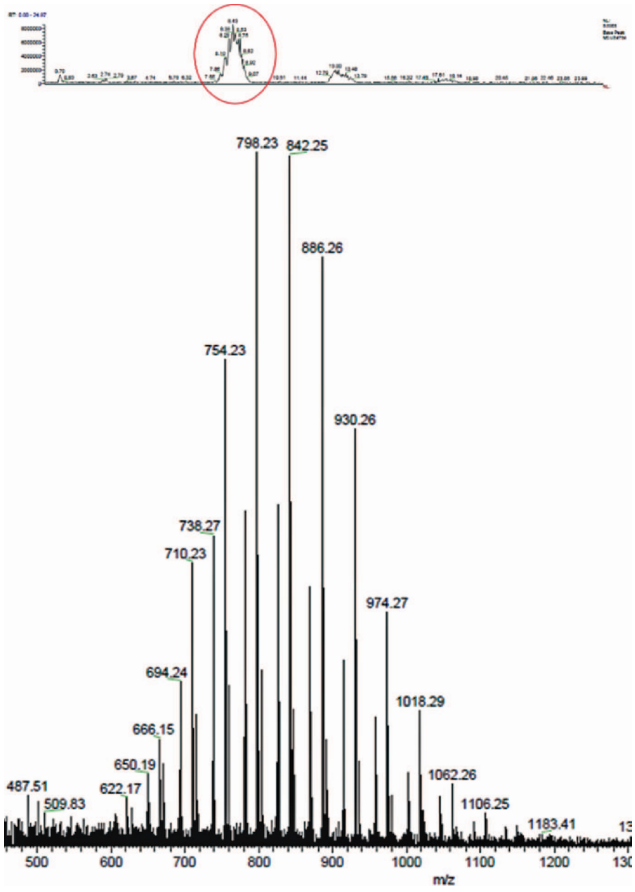

Figure 1: Upper part: Characteristic of the mass spectrum of the extracted solution. Lower part: Mass spectrum of the highest peak in detail.

numbers of the highest peak of the mass spectrum can be seen in detail. Figure 2 shows the mass numbers of the reference solution with the pure photopolymer monomers.

Due to the accordance of the mass numbers it could be proofed that the main component of the leachable substances are the monomers.

3.2 Cytotoxicity testing

The cytotoxicity tests showed that extracts of the cured photopolymer reduces the viability of cells by 20%, 26% and 29% after 3, 7 and 14 days of elution time (see Figure 3). Only after 30 days of elution time the cell viability is reduced by 45% and thus drops below the cytotoxicity level.

3.3 Genotoxicity of the photopolymer

As widely used in literature a 2-3 fold increase of bacteria growth of the probe compared to the negative control

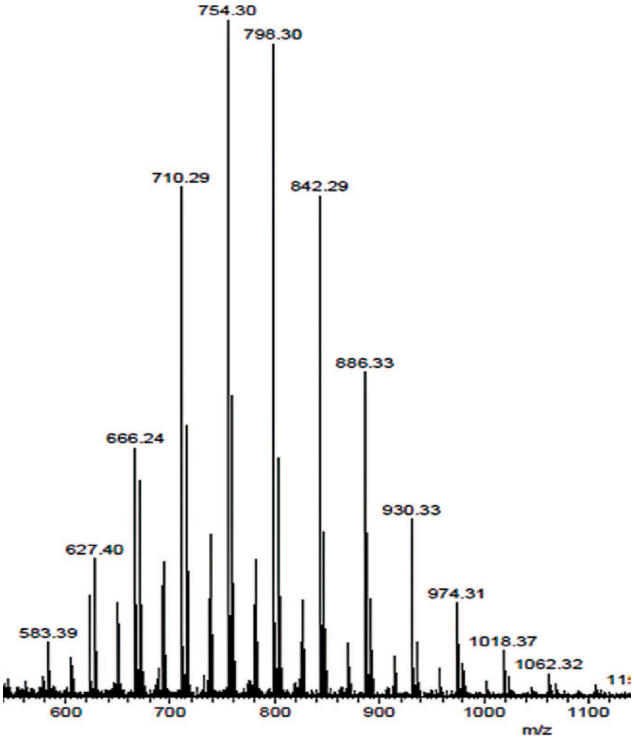

Figure 2: Mass spectrum of the reference solution with pure photopolymer monomers.

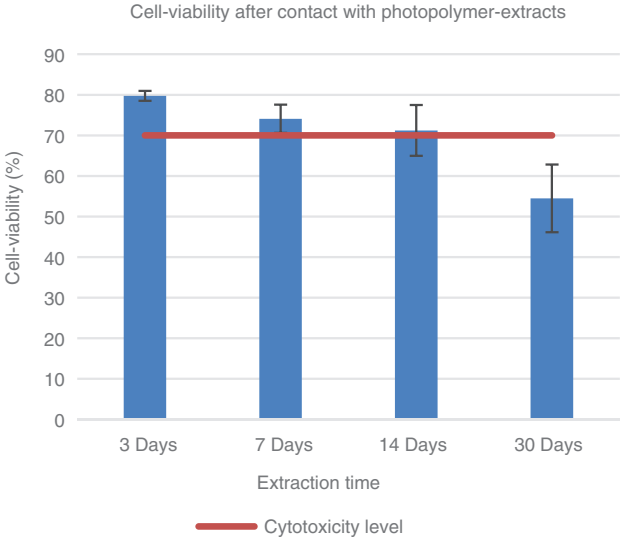

Figure 3: Cytotoxicity tests showed that the cured photopolymer induces no cytotoxic effect for usage up to 14 days. For usage up to 30 days a minor cytotoxic effect could be observed (n = 3).

was chosen as a cut-off between mutagenic and non-mutagenic response [9]. The tested photopolymer eluates showed no genotoxic effect up to an elution time of 30 days (see Figure 4).

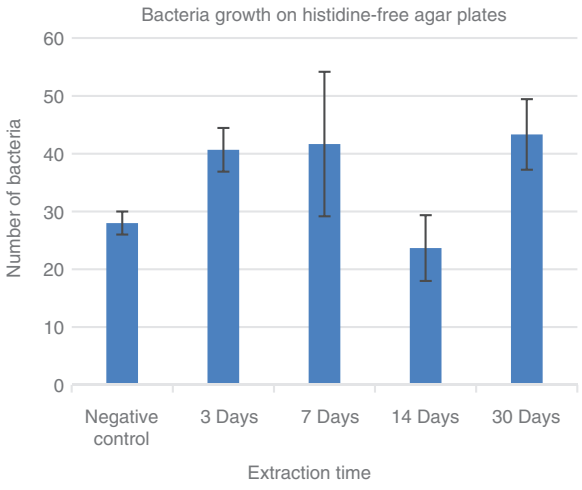

Figure 4: Genotoxicity tests showed that the cured photopolymer causes no genotoxic effect for elution times up to 30 days (n = 3).

4 Discussion

This study showed that the main component of the leachable substances out of the cured photopolymer are by far non-cured residual monomers, which therefore might have the greatest influence on the biocompatibility of the photopolymeric system. However, cytotoxicity tests showed that the cured photopolymer anyway can be considered as non-cytotoxic for elution times up to 14 days. For elution times of 30 days the photopolymer showed a minor cytotoxic effect, which might be caused by reaching a critical concentration of residual monomers. One option to further improve the biocompatibility is a post-processing of the cured photopolymer, like extraction of residual photopolymer monomers with supercritical CO_2. Another approach to reduce the amount of leachable substances would be the chemical modification of the chain length of the photopolymer monomers to reduce water uptake and therefore the dissolving of substances.

Genotoxicity tests revealed that there are no indications for mutagenic effects of the cured photopolymer.

5 Conclusion

In conclusion this study identified residual monomers as the presumable highest influence factor on the biocompatible properties of the photopolymeric system. Cytotoxicity tests revealed that the photopolymeric system GR-10 showed an only minor cytotoxic effect for very long elution times of 30 days, while for commonly used extraction times of up to 7 days no cytotoxic effect could be detected.

A mutagenic effect could not be observed. Therefore, GR-10 must be considered as a promising material for the production of class II or class III medical products on the basis of polymeric additive manufacturing, especially against the background that the release of residual monomers can be further reduced by simple to apply post-processing methods.

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Author's Statement

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