

Selina Schultz*, Anika Jonitz-Heincke, Rainer Bader, Niels Grabow, and Thomas Eickner

Manufacture and characterisation of PLLA films with incorporated cobalt metalparticles

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Abstract: To study the effects of metal ions on cells and tissues caused by wear and corrosion of implants in vitro, a system is required that releases metal ions in a controlled and sustained manner. As small concentrations can have an impact on biological systems, a correspondingly slow release over a defined period of time is required. A method for the manufacturing of polymeric poly(L-lactic acid) (PLLA) films with incorporated cobalt microparticles by dip coating is reported. Two analytical methods are established to demonstrate the detection and quantification of cobalt particles. Thermogravimetric analysis allows the quantification of cobalt particles. Scanning electron microscope images can be used to determine the distribution and number of particles of different size ranges.

Keywords: metal particles, wear, cobalt, PLLA films, TGA, DSC, SEM

1 Introduction

Metal implants for joint replacement due to trauma or osteoarthritis, such as knee or hip endoprostheses, are subject to permanent mechanical stress, which can lead to abrasion and the release of wear particles and ions as a result of physicochemical corrosion. This is one of the main causes of implant loosening and, as a result, implant failure. The contact of the implant surface with various tissues can lead to local inflammatory reactions, as an exchange of electrons and metal ions in various oxidation states takes place and oxygen radicals

(ROS) are produced. [1–3] Metal ions, such as cobalt, chromium, and nickel, can be released leading to adverse reactions in the surrounding tissues [3,4].

In order to analyse the release of metal ions from metal particles through contact with the surrounding medium, release systems consisting of a polymer matrix and embedded metal particles are required. In order to test the effect of metal ions in vitro, a release system with embedded particles is required. Various methods are already being used, e.g. incorporating micro- and nanoparticles into silicone [5] or PLLA [6] using solvent-based processes or into PLLA using extrusion [7].

Due to its biocompatibility, ability to absorb water and a degradation time of over two years, poly(L-lactic acid) (PLLA) is the polymer of choice to achieve a constant and uniform release of metal ions over the entire material volume (bulk) with almost no influence of the polymer within the first few weeks [7]. As a trace element, cobalt is essential for normal functioning of the body in low concentrations [1]. However, increasing cobalt content, for example as a result of particle abrasion from joint endoprostheses, can cause cardiological, endocrine or neurological toxicity [1,2].

In order to study the behaviour of cells and tissues towards metal ions under simulated physiological conditions, a controlled and sustained release of low concentrations over a defined period of time is required. Therefore, the aim of this study is to develop a method of manufacturing polymeric films with incorporated, homogeneously distributed metallic particles.

2 Materials and Methods

2.1 PLLA-cobalt film manufacturing

Poly(L-lactic acid) (PLLA) cobalt films are produced by dip coating as follows: a polymer solution containing 2 wt% PLLA (RESOMER L210, $M_w = 320.000$ g/mol, Evonik, Essen, Germany) is prepared in chloroform. Different masses of cobalt microparticles ($\varnothing = 2-4$ μm , ChemPUR, Karlsruhe,

*Corresponding author: **Selina Schultz:** Institute for Biomedical Engineering, Rostock University Medical Center, Friedrich-Barnewitz-Str. 4, 18119 Rostock, Germany, selina.schultz@uni-rostock.de

Thomas Eickner, Niels Grabow: Institute for Biomedical Engineering, Rostock University Medical Center, Friedrich-Barnewitz-Str. 4, 18119 Rostock, Germany

Anika Jonitz-Heincke, Rainer Bader: Department of Orthopedics, Rostock University Medical Center, Doberaner Straße 142, 18057 Rostock, Germany

Germany) are added to each 300 mL of polymer solution and homogenised using a mechanical stirrer, as shown in table 1.

Table 1: Resulting masses of cobalt in each PLLA film

PLLA film samples	1	2	3	4
Mass of cobalt [mg]	25	50	76	100
Weight percent [wt%]	1.64	3.28	5.00	6.58

In-house manufactured steel cylinders were coated by dipping into the PLLA-cobalt mixture for eight times and drying, resulting in a final film thickness of 110 μm , measured with a Mitutoyo Absolute device. Films were cut along the cylinder, peeled off and annealed at 80°C, under reduced pressure over 16 h in a vacuum chamber to obtain rectangular films (exemplarily shown in Fig. 1).

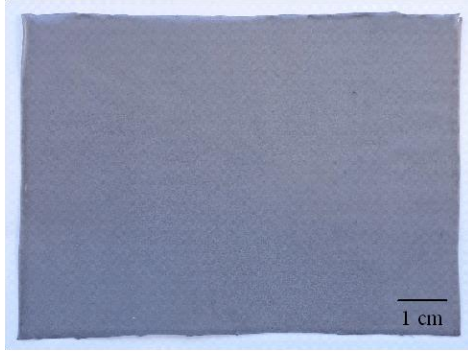


Figure 1: Film after eight cycles of dipping into polymer cobalt solution with 6.58 wt% cobalt and drying.

2.2 Determination of particle distribution using SEM

Morphology and particle-size distribution of the PLLA/cobalt dipped film samples were examined with SEM QUANTA FEG 250 (FEI Company, Dreieich, Germany) with an accelerating voltage of 5 kV and a proprietary spot size value of 4.0. Samples were cut from the film, embedded in epoxy, surrounded with conductive tape and images were taken at various magnifications. To analyse the particle distribution and number, the images were evaluated using ImageJ in terms of particle size and their distribution across the sample cross-section. For PLLA/cobalt, five images were analysed.

2.3 Thermal analysis using DSC

The thermal properties were analysed under nitrogen atmosphere using a DSC 1 star^e system (Mettler Toledo, Zurich, Switzerland). Sample pieces were cut from previously described materials and analysed in pure aluminum crucibles. The sample weights were in the range of 7-9 mg by stacking four samples on top of each other. The temperature programme used for all materials consists of the following steps: 1. cooling from room temperature to -30 °C and holding for 5 min, 2. heating to 200 °C and holding for 1 min, 3. cooling to -30 °C and holding for 5 min, 4. heating to 200 °C and holding for 1 min, 5. cooling to room temperature with a heating rate of 10 K/min and a cooling rate of 5 K/min. During the first heating process, the influences of manufacturing processes are to be determined. As a result of heating to 200°C, the thermal history of the material is erased and a reference of the material is measured during the second heating [8]. Thermograms were analyzed with respect to glass transition (T_g), melting temperature (T_m) and degree of crystallinity (χ). Heat of fusion and crystallinity were quantitatively evaluated by comparing the determined endothermic peak areas with the melting peak areas of totally crystalline PLLA ($\chi_{100} = 93.7 \text{ J/g}$) [7]. For each composite, $n = 2$ measurements were performed.

2.4 Determination of mass loss using TGA

The thermogravimetric analysis was carried out in a TGA/DSC3+ star^e system (Mettler Toledo, Zurich, Switzerland). Sample were prepared as described (2.2). The measurements were carried out in open aluminum crucibles by heating from 50 °C to 550 °C at a heating rate of 10 K/min with a sample weight of approx. 10 mg. For each composite, $n = 2$ measurements were performed. Before measurements, a blank curve was performed without a crucible using the same temperature programme. The base mass (m_{BL}) from the blank curve was mathematically subtracted from the measurement mass (m_{meas}) and the thermograms were then analysed with regard to the melting and decomposition temperature (T_m / T_d) and the percentage mass loss [%] according to equation (1).

$$m_{rel} = m_{meas} - m_{BL} \quad (1)$$

3 Results and Discussion

In order to prevent the heavy metal particles from sinking and thus avoiding an inhomogeneous particle distribution, the films were produced using a dipping process.

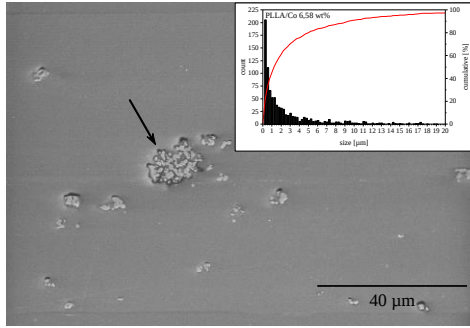


Figure 2: SEM image of the cross-section with visible Co-particles (arrow) and histogram for particle count for PLLA/6.58 wt% cobalt.

Figure 2 shows an SEM image of the distribution of the microparticles over the entire cross-section of the approx. 110 μm thick sample with an included histogram of the number of particles for a sample of PLLA/6.58 wt% cobalt. A statement about the homogeneity of the particle distribution is only possible by analysing several SEM images in different areas of the films over different layers. However, it is important to mention that the evaporation process of the solvent during immersion is sufficiently fast and the particles do not sink. However, agglomeration of the particles can be observed.

The curves in Figures 3 and 4 show representative measurement curves for pure PLLA and PLLA/6.58 wt% cobalt using DSC and TGA. Table 2 summarises the measured values for the characteristic temperatures (T_g , T_c , T_m , T_d), crystallinity (χ) and mass loss.

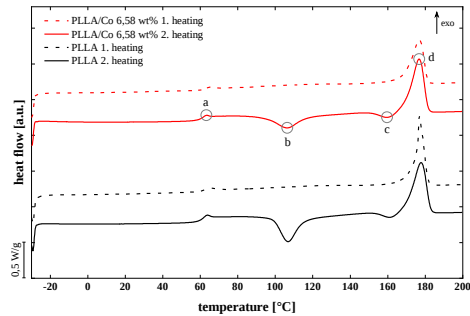


Figure 3: Thermogram of the first and second heating curve for pure PLLA and PLLA/6.58 wt% cobalt with glass transition (a), cold crystallisation (b), transformation α' to α -crystal (c) and melting range (d).

The DSC thermogram shows characteristic curves, starting with the glass transition at 60 °C (peak a), the following cold crystallisation during the second heating at 110 °C (peak b), the transformation from α' to α -crystal at 160 °C (peak c) and the subsequent melting temperature at 180 °C (peak d). The first and second heating of the materials differ mainly in terms of peak b. This is due to the fact that during the drying process while manufacturing, annealing took place, which makes the material thermodynamically stable. By heating, cooling and reheating, the thermal history of the material is erased, which is why cold crystallisation now occurs, as would be the case with thermally untreated material. Except for the decomposition temperature, the thermal properties are not significantly influenced by the addition of cobalt probably because of thermodynamic stability.

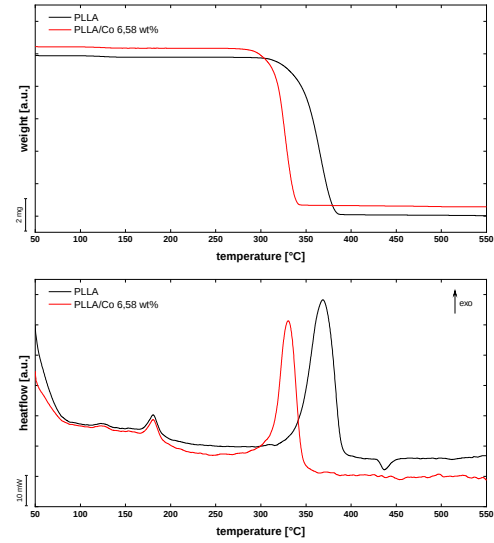


Figure 4: Thermogravimetric curve of pure PLLA and PLLA/6.58 wt% cobalt with melting point (T_m) and decomposition temperature (T_d)

Table 2: Thermal properties and mass loss for pure PLLA and PLLA/cobalt composites (n=2)

	PLLA	PLLA/cobalt			
		1.64 wt%	3.28 wt%	5.00 wt%	6.58 wt%
T_g [°C]	61.1 ± 0.7	61.6 ± 0.1	61.9 ± 0.2	67.1 ± 0.9	61.5 ± 0.1
T_m [°C]	177.8 ± 0.3	177.8 ± 1.1	178.3 ± 0.3	178.4 ± 0.6	177.7 ± 0.2
χ [%]	44.7 ± 0.2	44.9 ± 9.3	42.4 ± 0.3	39.8 ± 2.0	43.1 ± 0.8
T_d [°C]	369.3 ± 0.5	343.7 ± 0.4	336.3 ± 1.0	334.9 ± 0.3	330.4 ± 0.5
mass loss [%]	98.9 ± 0.6	97.7 ± 0.2	95.8 ± 0.5	94.9 ± 0.5	92.9 ± 0.3

The single-step TGA curve showed an onset of mass loss for pure PLLA at 310 °C and for PLLA/6.58 wt % cobalt at 280 °C. The mass loss that starts at lower temperatures for PLLA/cobalt is also reflected in the exothermic peak of the decomposition temperature in the DSC curves in Figure 4. The shift of the decomposition peak in cobalt-containing samples could be due to polymer chains scission due to the manufacturing process with resulting thermal instability of the PLLA or heat transfer to PLLA promoted by the increased thermal conductivity of cobalt [7]. The mass loss determined using TGA approximately reflects the values obtained for the cobalt content (table 2).

The curves from DSC and TGA and measured values clearly show that the presence of cobalt particles only has an influence on the decomposition temperature of the material except for PLLA/5.00 wt% cobalt, which is considered an outlier.

4 Conclusion

In this experimental study, a method for the manufacturing of PLLA films with incorporated cobalt particles is presented. Two analytical methods, SEM and TGA, were used to determine the content and number of particles in the polymer, respectively. The TGA showed that the incorporation of cobalt particles does not lead to any change in the thermal properties, except for the decomposition temperature. Furthermore, it could be shown that the actual cobalt content is close to the value obtained. The analysis of SEM-images makes it possible to determine the number of particles of different size classes and to give information about the homogeneity of the samples and agglomerations of particles.

Our methods used avoid structural changes in the particles due to chemical stimuli and can be transferred to other polymers and metal particles to enable systematic studies on the toxicity of metal ions derived from nanoparticles on different cell types and tissue.

Author Statement

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Conflict of interest: Authors state no conflict of interest

References

- [1] Badhe RV, Akinfosile O, Bijukumar D, Barba M, Mathew MT. Systemic toxicity eliciting metal ion levels from metallic implants and orthopedic devices - A mini review. *Toxicology letters* 2021;350:213–224.
- [2] Bijukumar DR, Segu A, Souza JCM, et al. Systemic and local toxicity of metal debris released from hip prostheses: A review of experimental approaches. *Nanomedicine : nanotechnology, biology, and medicine* 2018;14:951–963.
- [3] Matusiewicz H. Potential release of in vivo trace metals from metallic medical implants in the human body: from ions to nanoparticles--a systematic analytical review. *Acta biomaterialia* 2014;10:2379–2403.
- [4] Hahn A, Fuhlrott J, Loos A, Barcikowski S. Cytotoxicity and ion release of alloy nanoparticles. *Journal of nanoparticle research : an interdisciplinary forum for nanoscale science and technology* 2012;14:1–10.
- [5] Hahn A, Brandes G, Wagener P, Barcikowski S. Metal ion release kinetics from nanoparticle silicone composites. *Journal of controlled release : official journal of the Controlled Release Society* 2011;154:164–170.
- [6] Kurzina IA, Laput OA, Zuza DA, et al. Surface property modification of biocompatible material based on polylactic acid by ion implantation. *Surface and Coatings Technology* 2020;388:125529.
- [7] Oksiuta Z, Jalbrzykowski M, Mystkowska J, Romanczuk E, Osiecki T. Mechanical and Thermal Properties of Polylactide (PLA) Composites Modified with Mg, Fe, and Polyethylene (PE) Additives. *Polymers* 2020;12.
- [8] Anderson KS, Hillmyer MA. Melt preparation and nucleation efficiency of polylactide stereocomplex crystallites. *Polymer* 2006;47:2030–2035.