

Analysis of the aging of cell-adhesive plasma-polymer coatings on titanium

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A crucial factor for titanium implant performance is the rapid acceptance of osteoblastic cells just after implantation. It could be shown that the aging of microwave plasma deposited allylamine polymer films on polished titanium surfaces has no influence on cell adhesion and spreading.

Keywords: Pulsed plasma polymerization, amino functionalization, thin layer, aging, cell behavior

1. Introduction

Plasma polymer deposition is the method of choice for the finishing of metallic implant materials like titanium with nitrogen-containing bioactive coatings. The deposited cell-adhesive plasma polymer films have to possess special properties such as homogeneity, film stability on air as well as in different media, sufficient density of functional groups and the appropriate surface charge. But also the knowledge of long-term stability is essential for the application as implant surface. Therefore, aging studies of plasma polymer coatings on titanium surfaces are important to detect the changes of surface chemistry over a longer time period. For this purpose, results of physicochemical surface diagnostics were combined with adequate tissue culture experiments. The objective of this paper was to measure surface chemical characteristics of thin plasma polymerized allylamine (PPAAm) coatings on polished titanium with the main focus on FT-IR studies over a time period of one year and to correlate these data with the adhesion of human MG-63 osteoblastic cells.

2. Materials and Methods

Polished titanium disks (Ti-P) ($R_a=0.4\ \mu\text{m}$), 11 mm in diameter were used as substrates for the chemical functionalization. The preparation was carried out in two steps:

- (1) Disks were decontaminated and activated by cw oxygen plasma, followed by
- (2) Plasma polymerization of the monomer allylamine using a micro-wave (MW) excited (2.45 GHz, 500 W) pulsed (duty cycle of 0.15 at a pulse length of 2 s) low pressure ($p = 50\ \text{Pa}$) gas-discharge plasma for 144 s [1, 2].

The elemental chemical surface composition and chemical binding properties were determined by high-resolution scanning X-ray photoelectron spectroscopy (XPS). The Axis Ultra spectrometer (Kratos, UK) runs with the monochromatic Al K_α line at 1486 eV (150 W)

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implemented charge neutralization and a pass energy of 80 eV for estimating the chemical elemental composition or 10 eV for highly resolved C1s spectra. The C-C/C-H component of the C1s peak was adjusted to 285.0 eV. Amino groups were labelled by reaction with 4-trifluoromethyl-benzaldehyde (TFBA) at 40 °C in a saturated gas phase for 2 h [1].

Additionally, the PPAAm-films were investigated by Fourier-Transform-Infrared Spectroscopy (FT-IR) using the diamond attenuated total reflection (ATR)- or the IR-reflection-absorption spectroscopy (IRRAS)- unit of a Spectrum One (Perkin-Elmer, Germany) spectrometer.

Water contact angles were measured by the sessile drop method (drop volume ~0.5 µl) using the measuring system OCA30 (Data Physics Instruments GmbH, Germany).

Human osteoblastic cells (MG-63 cells, ATCC) were cultured under serum-free conditions to avoid masking of the amino-functionalized Ti-P surfaces with adsorbed proteins [1, 2].

3. Results and Discussion

As expected, XPS and FT-IR (ATR and IRRAS) measurements demonstrate the oxidation of the PPAAm film by post plasma processes [3], initiated by surface free radicals during sample storage on air. XPS elemental analysis showed a closed pinhole-free polymer film since no Ti signal was observed. The N/C ratio was found to be at 27.2±0.2 % (19.2±0.5 %) and O/C at 5.6±0.4 % (16.2±0.3 %), measured after preparation and one year's storage (in brackets). The theoretical value for the precursor allylamine is N/C=33.3 %, no O/C. Amino groups, determined from the XPS elemental ratio F/C, are present with a density of about 2.5±0.5 % (0.20±0.07 %), after preparation and one year's storage (in brackets). About 70 % of primary amino groups have been lost within the first 30 days of storage, while the nitrogen content remains nearly stable. The loss of primary amino groups (CF₃-peak at 292.7 eV) is accompanied by an oxidation to acid amides at 288.2 eV and increasing oxygen functional groups (C-O, C=O peaks at 286.6 eV and 287.5 eV).

The PPAAm surface shows remarkable amounts of -C-NH₂ bonds and thus can counterbalance the existing negatively charged oxygen functional moieties. This should lead to a positive surface charge in aqueous environment. The zeta-potential of a freshly prepared [1,4] and also an aged PPAAm surface was found to be slightly positive [4].

FT-IR studies confirmed a high retention of the structural properties of the monomer allylamine [5] for the deposition method used here. Basic structures of the monomer are dominant: the stretching vibrations of the aliphatic C-H groups, ν -CH_{2,3} at 2980-2880 cm⁻¹, the deformation vibration, δ -CH_{2,3} at 1465-1375 cm⁻¹, and deformation vibrations of amines, δ -NH at 1650-1510 cm⁻¹. Also ν -NH stretching vibrations between 3380-3200 cm⁻¹ are

clearly visible. Typical plasma and also aging effects can be observed as e.g. significantly broadened, disappearing or arising bands. Here, amino groups were partially transformed into amide, imine or nitrile functional groups by the plasma process. An indication for that is the band between $2300\text{-}2200\text{ cm}^{-1}$ associated with stretching vibrations of nitrile and ethine groups, $\nu\text{-CN}$, $\nu\text{-CC}$. FT-IR spectra of the PPAAm-surface after preparation and aging on air for 7 days are compared in **Fig. 1**.

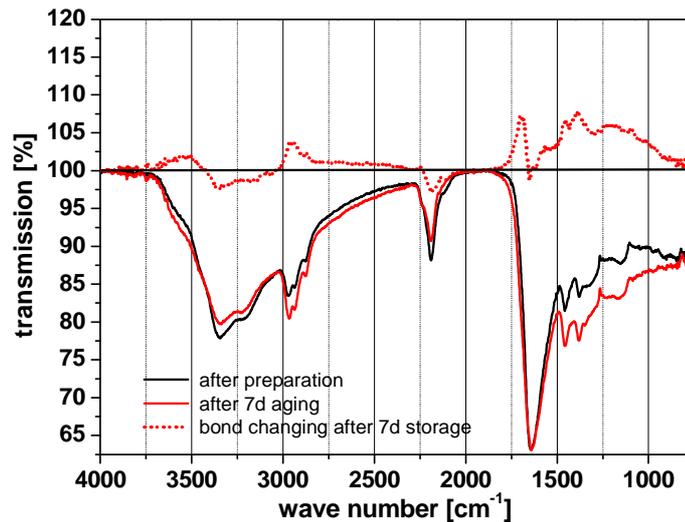


Figure 1: FT-IR ATR spectra of PPAAm surfaces after preparation and storage on air over 7 days

Characteristic changes due to ageing already after 7 days (dotted graph in Fig. 1) are: the formation of O-H vibrations near $3700\text{-}3200\text{ cm}^{-1}$ by water absorption, the formation of acid amides at $1700\text{-}1680\text{ cm}^{-1}$ [6], a degradation of nitrile and ethine groups, $\nu\text{-CN}$ and $\nu\text{-CC}$ at about $2200\text{-}2150\text{ cm}^{-1}$.

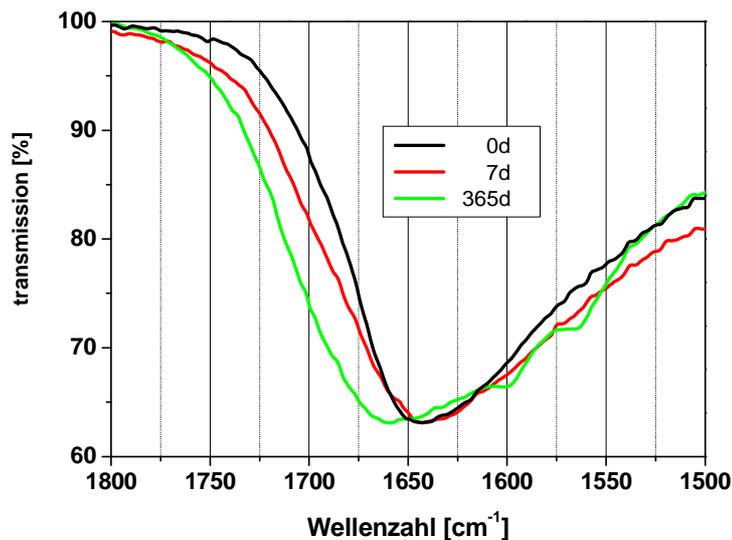


Figure 2: FT-IR IRRAS spectra of PPAAm surfaces after preparation and storage on air up to 365 days

The analyses of the v-N-H vibration band around $1750\text{-}1500\text{cm}^{-1}$ shows the broadening by the oxidation process, the shifting of this band and also substructures during the aging process. First signs for the broadening can be observed after 7 days (Fig. 2) already.

Different water contact angle were detected (PPAAm $48\pm 3^\circ$, untreated Ti-P $87\pm 3^\circ$). The water contact angle for PPAAm decreased to $36\pm 2^\circ$ after 360 days storage on ambient air. Thus, advantageous medium hydrophilicity still exists. This means that the wettability of PPAAm covered titanium is optimum for cell adhesion even after one year storage [7].

Cell culture experiments with MG-63 human osteoblast-like cells demonstrated a significantly enhanced adhesion and spreading on a PPAAm coated Ti-P substrate not only immediately after preparation as already observed [1,2,4, 8-10] but also independent of the storage duration up to one year.

In fact, not only primary amino groups but also other N-functional groups as e.g. acid amides or imides seem to play a role for initial cell functions [11]. Furthermore the examined plasma polymer coating PPAAm enables the cells to exhibit a very flattened and widespread phenotype indicating a strong cell-surface contact for improved implant ingrowth. Our newest results demonstrate that this long-time stable PPAAm-nanolayer has an impressive impact versus the titanium surface topography on osteoblast orientation [12].

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