**Supplemental material for**

# Potential ecotoxicological effects of antimicrobial surface coatings: A literature survey backed up by analysis of market reports.

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## Referenced results from the literature survey for Ti, Cu, Zn, chitosan and QAC containing surfaces

### *Titanium containing coatings*

For titanium-based AMCs, 165 papers were retrieved with the “release” search. However, 55% of these papers concerned materials that were deposited on titanium or titanium alloy substrates. Out of papers where titanium was used as an antimicrobial ingredient, only four (2.4%) studied the amount of released Ti: no titanium was released from stainless steel intramedullary nails coated with titanium oxide and siloxane polymer doped with Ag neodecanoate into goat serum samples (Gladitz, Reinemann & Radusch, 2009), no leaching of titanium ion from the hetero-nanostructured multilayer films comprising negatively charged titania nanosheets and positively charged lysozyme into phosphate-buffered saline was observed (Wang & Zhang, 2012), Ti release was negligible from Ti6Al4V substrates and the same substrates coated with a thin film of Si/F (Santos-Coquillat et al., 2018), and only Godoy-Gallardo et al. (2016) reported trace amounts of titanium in tissues around dental implants. More often the release of other substances from Ti substrates or Ti-containing coatings was studied. For example, 34 papers, included in the previous chapter where silver had been used together with titanium, the release of Ag ions was studied. In coatings where a combination of titanium with other metals was used, release of Cu (Finke et al., 2012; Wei et al., 2014a; Peres et al., 2014; Hempel et al., 2014; Chen et al., 2014a), Zn (Huang et al., 2017), and Mg (Zaatreh et al., 2017) was also reported. Titanium coatings have been also combined with antibiotics or other antimicrobials where release of vancomycin (Swanson, Cheng & Friedrich, 2011; Pitarresi et al., 2013; Wang et al., 2016; Han et al., 2017; Bakhshandeh et al., 2017), gentamicin (Moskowitz et al., 2010; Pérez-Anes et al., 2015; Hizal et al., 2015; Wang et al., 2017b), minocycline (Darouiche, 2007; Lv et al., 2014), rifampin (Kälicke et al., 2006; Darouiche, 2007), tetracycline (Cai et al., 2016; Bottino et al., 2017), ciprofloxacin (Mattioli-Belmonte et al., 2014) and fusidic acid (Kälicke et al., 2006); antiseptics like chlorhexidine (Harris et al., 2006; Chang et al., 2014; Wood et al., 2015), octenidin and irgasan (Kälicke et al., 2006); antimicrobial peptides like catestatin (Özçelik et al., 2015), recombinant human b-defensin-2 (rHubD2)(Pfeufer et al., 2011), Cationic Steroidal Antimicrobial‐13 (CSA‐13) (Sinclair et al., 2012), cov-GL13K (derived from the human salivary protein Parotid Secretory Protein (BPIFA2)) (Chen et al., 2014b); or other molecules like cyclodextrin (Pérez-Anes et al., 2015) and butenolide (Chen, Xia & Qian, 2017), was studied.

### *Copper containing coatings*

Release from copper-containing AMCs was mentioned in 122 documents, with only 36 results (30%) containing information on release of some substances. Among these, 32 papers had data on copper release. Besides copper, also (co-)release of other substances that were used in combination with copper was investigated from copper-containing surfaces: silver (Yeasmin et al., 2016; Harrasser et al., 2016; Haase et al., 2017; Ciacotich et al., 2018), zinc (Watermann et al., 2005; Sathya et al., 2016; Ytreberg et al., 2017), lead (Watermann et al., 2005), chromium (Watermann et al., 2005), cadmium (Watermann et al., 2005), nickel (Watermann et al., 2005), organotin compounds (Thomas et al., 1999; Watermann et al., 2005), hydrogen peroxide (Olsen et al., 2010), diuron (Faÿ et al., 2018), tolylfluanid (Faÿ et al., 2018), copper thiocyanate (Faÿ et al., 2018), 4,5-dichloro-2-n-octyl-3(2H)-isothiazolone (Sea Nine®[Rohm Haas]) (Howell & Behrends, 2006), nonylphenol (Watermann et al., 2005), bisphenol A (Watermann et al., 2005), rosin (Peres et al., 2014). Marine (15 of 36 papers, 42%) and medical/general purpose (18 of 36 papers, 50%) applications were more or less equally represented, when special applications such as reverse osmosis membranes (Ben-Sasson et al., 2014), water filtration systems (Padmavathy et al., 2017) or photo-excited outdoor applications (Wei et al., 2014a) were not accounted for. Influence of cleaning on active ingredient release and degradation of coatings has been rarely studied. Namely, only two cases were found for copper-containing AMCs: abrasive cleaning of marine coating (Schiff, Diehl & Valkirs, 2004) and repeatedly wiping copper-loaded chitosan/silica surfaces with water (Mitra et al., 2017). Degradation of the surface was studied in one paper as rosin dissolution in non-marine application (Wei et al., 2014b).

Copper release rate from "Lacomit"lacquered pure copper surface was about 160 μg/cm2 per day after 2 weeks and 70 μg/cm2 per day after 18 weeks of static incubation in seawater and less for copper alloys (Hall & Baker, 1985, 1986). For copper-based marine antifouling coatings, either commercial or experimental, stable release rates after a few weeks were from under 1 μg/cm2 per day (~4 weeks (Sathya et al., 2016)) to ~20-30 μg/cm2 per day in different periods (2.5 weeks (Thomas et al., 1999); 4 weeks (Howell & Behrends, 2006); ~12 weeks (Valkirs et al., 2003)). As for silver-based AMCs, also in copper containing AMCs, copper release was experimentally lowered for example by using Cu nanowires in elastomeric polydimethylsiloxane (PDMS) (stable release ~35 ng/cm2 per day after ~1.5 weeks and less than 2 % of total copper on the surface was released during 50 days (Jiang et al., 2015)) or by exploiting using hydrophobic nanocomposite films (Sathya et al., 2016). Control strategies to prolong the release also included crosslinking (Padmavathy et al., 2017) or encapsulation of active ingredients (Liu et al., 2017). Abrasive cleaning of surfaces painted with commercial antifouling paints containing cuprous oxide increased short-term release rate for about 3-fold for a few days after which the release dropped to baseline long-term release rate (Schiff, Diehl & Valkirs, 2004). Compared to slow-releasing marine applications, surfaces with proposed medical or other specific or general antibacterial surface application generally demonstrated burst release profile and high release for first hours or days of the experiment rather than stable release for weeks or months. For example, Taglietti et al. (2018) showed total Cu depletion at physiological pH already in 5 hours, Ben-Sasson et al. (2014) demonstrated 30% Cu depletion during 24 h from reverse osmosis membranes, and Rubin, Neufeld & Reynolds (2018) reported 54-90% Cu depletion from textile during 24 h. Different cleaning protocols potentially greatly influence efficiency of non-invasive AMCs but are rarely investigated regarding release and depletion of active ingredient. For example, wiping copper-loaded acrylated quaternized chitosan/silica on poly(vinyl fluoride) films with deionized water moistened paper resulted in no decrease to 67% decrease in Cu content after 60 wipes and 68%-90% decrease in Cu content after 100 wipes, depending on specific surface architecture (Mitra et al., 2017).

### *Zinc containing coatings*

Zinc, in the context of release from AMCs was mentioned in 66 articles with 27 papers (41%) containing at least some kind of data on release. Among these, only 17 had (semi‑)quantitative data for zinc release. Besides zinc, also (co-)release of other substances used in combination with zinc was investigated from zinc-containing surfaces: silver (Coughlan et al., 2008; Agnihotri et al., 2015; Kumar S. Mural et al., 2016), copper (Watermann et al., 2005; Sathya et al., 2016; Ytreberg et al., 2017), hydrogen peroxide (Olsen et al., 2009; Kristensen et al., 2010), crystal violet (Noimark et al., 2015; Ozkan, Allan & Parkin, 2018), paeonolsilate (Sun et al., 2016; Yang et al., 2017), salicylate (Bellotti et al., 2013; Bellotti & Romagnoli, 2014), magnesium (Zaatreh et al., 2017), vanadium (Zhou et al., 2016), lead (Watermann et al., 2005), chromium (Watermann et al., 2005), cadmium (Watermann et al., 2005), nickel (Watermann et al., 2005), organotin compounds (Watermann et al., 2005), chlorine dioxide (Li et al., 2009), chlorhexidine (Carteau et al., 2014), butenolide (Chen, Xia & Qian, 2017), benzoate (Bellotti et al., 2013), polyphenols (Bellotti, del Amo & Romagnoli, 2012a), chitosan (Al-Naamani et al., 2017), nonylphenol (Watermann et al., 2005), bisphenol A (Watermann et al., 2005). Cleaning or reusing the surfaces was generally not explored with a couple of exceptions e.g., washing ZnO NP-containing textile (Manna et al., 2013) or repeated use of Ag/ZnO nanomaterial-coated surfaces (Agnihotri et al., 2015). Material degradation was rarely evaluated except for marine applications where degradation is described as thickness or polishing rate of the coating and not measured as release of degradation products. Marine antifouling coating was proposed application in 13 (48%) of the 27 studies including specific material architecture and release study conditions with stable long-term releases up to 100 μg/cm2 per day (Mu et al., 2017). Less than half of the papers proposed medical applications, from invasive device coatings to more general surfaces that could be used in healthcare settings or other public areas. Surfaces for medical applications generally demonstrated burst release profile with for example 85-96% Zn depletion in 24 h (Malzahn et al., 2013). Slower release rates were achieved by using zinc-incorporated chitosan/gelatin nanocomposite coatings (10-20% Zn loss in 4 weeks) (Huang et al., 2017).

### *Chitosan containing coatings*

Release from chitosan-based AMCs was documented in 74 papers, out of which 26 results (35%) contained actual release data. Among these, in 6 papers chitosan degradation and/or possible release of degradation products was evaluated (Huang et al., 2011; Avetta et al., 2014; Pérez-Anes et al., 2015; Ma et al., 2015; Mitra et al., 2016; Al-Naamani et al., 2017). In most studies (12 papers), chitosan was used as a reservoir for other antimicrobials: vancomycin (Swanson, Cheng & Friedrich, 2011; Bakhshandeh et al., 2017), gentamicin (Ma et al., 2015; Xu et al., 2017), ciprofloxacin (Avetta et al., 2014; Mattioli-Belmonte et al., 2014), tetracycline (Cai et al., 2016), cefepime (Pebdeni, Sadri & Pebdeni, 2016), minocycline (Lv et al., 2014), β-peptide (Raman et al., 2016), berberine (Huang et al., 2011), triclosan (Wang et al., 2017a). In 10 papers ionic metal release was recorded: silver (Pang & Zhitomirsky, 2008; Travan et al., 2011; Li et al., 2013a; Nie et al., 2017; Bakhshandeh et al., 2017; Cometa et al., 2017; Guo et al., 2018); copper (Abiraman & Balasubramanian, 2017; Mitra et al., 2017); zinc (Huang et al., 2017). In a few cases, also non-antimicrobials e.g., toluidine blue ortho (TBO) (Pérez-Anes et al., 2015), bovine serum albumin (BSA) (Ma et al., 2015), anticancer drug doxorubicin (Ma et al., 2015) or liquid smoke (Ceylan et al., 2017) were incorporated into chitosan surface coatings. Effect of cleaning was investigated in only one paper (Mitra et al., 2017).

Most of the studied surfaces presented coatings with high initial burst release profile during first hours up to 1-2 days of release experiment followed by period of residual slow release of variable length depending on surface material properties and initial loading amount of substances released from the surfaces. Strategies used to control the release of active compounds were either passive via extending the release in time and/or flatten out initial burst in time by entrapping active substances using mostly layered systems and/or crosslinking (Pang & Zhitomirsky, 2008; Lv et al., 2014; Pérez-Anes et al., 2015; Ma et al., 2015; Raman et al., 2016; Mitra et al., 2016; Nie et al., 2017; Xu et al., 2017) or active control by pH-triggered release of antimicrobial substance (Wang et al., 2017a).

### *Coatings containing quaternary ammonium compounds*

57 articles mentioned QACs in the context of release from AMCs to which QACs were either covalently crosslinked (33 articles), deposited (6 articles), mixed (2 articles), grafted, immobilized, impregnated (all 1 article), or introduced by another method. In 18 articles, release of QACs from surfaces was not discussed at all, while 20 (35%) articles out of 57 contained certain indicative information that could be used to assess the stability of surfaces. For example, Liu et al. (2015), He et al. (2016), Yan et al. (2017), Sugii et al. (2017), and Cuervo-Rodríguez et al. (2017) assumed no potential QAC leakage. Their conclusion was based on the chemical properties of the used QACs or due to their covalent linking with the surfaces. Another group of articles (Yang et al., 2012; Farah et al., 2013; Michailidis et al., 2017) tested the ability (and thus, indirectly also the leakage of QACs) of surfaces to retain their antimicrobial properties after storage e.g., in seawater (Yang et al., 2012; Michailidis et al., 2017), phosphate-buffered saline (Beyth et al., 2008), deionized water (Church et al., 2017), bacterial growth media (Saini et al., 2016) or detergent-containing water (Saif et al., 2015) after specified storage time ranging from weeks till 5 months, or after significant number of washes using detergent-containing water (e.g., QAC-containing textiles in Saif et al. (2015)). In all these studies, except in the Church et al. (2017), antimicrobial effect of coatings was retained during storage or after washing, and no sign of its decrease was observed. Only Church et al. (2017) observed release of QAC-containing NPs from antimicrobial concrete surface whereas the proof for QAC NP (Fixed Quat) release was antimicrobial activity of wash water. In addition to testing of the antimicrobial activity of aged QAC surfaces, some articles also evaluated leaching of QACs by using zone of inhibition (ZOI) test (Lee et al., 2004; Kurt et al., 2007; Beyth et al., 2008; Yagci et al., 2011a,b; Asri et al., 2014; He et al., 2015; Zhao et al., 2015). In these assessments, the QAC containing surfaces were placed as small discs onto agarized surface (rich growth medium or blood containing agar on which the microbial suspension was spread) and zone of inhibition of bacterial growth around the discs was analyzed. In none of these papers ZOI was observed, i.e. no leakage of QACs was concluded. Another group of papers investigated the stability of QAC surfaces by leaching the surfaces and subsequently testing the antimicrobial effects of leachates (Han et al., 2011; Jellali et al., 2013; Asri et al., 2014; Church et al., 2017). In none of these studies, the tested leachate exhibited antimicrobial effect. Only 6 (11%) articles out of 57 measured the release of QACs or quantified the QAC release. Chen et al. (2017) followed the amount of chlorine as a proxy for QAC content on surfaces. They observed that during single leaching with 0.15% AATCC detergent solution, 65% of antibacterial components were removed, while removal was 67% or 80% with 3 and 5 washing cycles, respectively. Such a decrease of antibacterial components did not render the surfaces ineffective as even after 10 washing cycles the surfaces stayed antibacterial (as 0.05 wt% QAC, a threshold antibacterial concentration, was still present on the surfaces). Pérez-Köhler et al. (2016) used HPLC to follow chlorhexidine release from polypropylene surfaces. From these surfaces, to which the QAC was bound electrostatically, 20% of chlorhexidine was released during first hour, ~30% was released during 5 h, ~ 40% during 24 h, ~55% during 48 h, and 100% of chlorhexidine was released during 72 h incubation in phosphate buffered saline. In another study, Bellotti, del Amo & Romagnoli (2012b) demonstrated the QAC release from paints based on rosin/oleic acid and rosin/polystyrene by analyzing the amount of QAC-associated polyphenols. In seawater, from a surface with initial content of 1.5 g QAC/cm2, 1-3.5 µg/cm2 (thus, 0.0001-0.0003% of the initial material) was released in one day. A study by Coneski, Fulmer & Wynne (2012) quantitatively assessed the hydrolysis of degradable polymer to which quaternary ammonium salt was added. In distilled water, 8-25% of polymer was degraded within 4 weeks and 10-35% of the polymer within 16 weeks. In two studies (Wei et al., 2016, 2017), QAC containing surfaces were specifically designed for triggered release of the biocidal agent. In the earlier study, Wei et al. (2016) demonstrated destruction of β-cyclodextrin attached to surfaces via electrostatic forces by use of surfactants such as SDS. Indeed, most of the β-cyclodextrin was removed from surfaces after SDS treatment. Later, Wei et al. (2017) used UV light at 365 nm 10 mW/cm2 to trigger the QAC release and demonstrated that 30 min of exposure to UV was enough to release 89.6% of the used azobenzene-quaternary ammonium salt complex.

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