Pro-Antimicrobial Networks via Degradable Acetals (PANDAs) Using Thiol–Ene Photopolymerization

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Supporting Information

ABSTRACT: We describe the synthesis of pro-antimicrobial networks via degradable acetals (PANDAs) as a new paradigm for sequestration and triggered release of volatile, bioactive aldehydes. PANDAs derived from diallyl p-chlorobenzaldehyde acetal degrade and release p-chlorobenzaldehyde as an antibacterial and antifungal agent under mild conditions (pH 7.4/high humidity). We show that PANDAs enable facile access to materials with tunable release profiles, potent antimicrobial activity without triggering antimicrobial resistance, and minimal cytotoxicity.

With 700000 annual deaths globally, antimicrobial resistance is an escalating crisis that threatens the sustainability of public health and agricultural ecosystems. As the effectiveness of antibiotics has precipitously declined, a growing interest in alternative antibiotic scaffolds has proliferated. In this direction, aromatic terpene aldehydes, major phytochemical constituents of plant derived essential oils (EOs), are known to exhibit potent and broad spectrum antibacterial and antifungal activities in both liquid and gaseous systems; however, these strategies often suffer deficiencies such as low loading, poor encapsulation efficiencies, necessity of organic processing solvents, and uncontrolled burst release profiles.

“Polyactives”, polymeric pro-drugs that undergo degradation to release active therapeutic agents, address the deficiencies in sequestering EO constituents by providing high loading, chemical stability, and tailored release kinetics. Recently, linear polyactives of phytochemicals and other synthetic analogs have been used for the extended delivery of antimicrobials, antioxidants, anti-inflammatory agents, and anticancer therapeutics. While successfully demonstrated, tuning the degradation of linear polyactives remains a challenge as degradation rates are determined by manipulating coupled parameters such as crystallinity, polymer molecular weight, functionality, and hydrophathy. Alternatively, cross-linked polyactives (i.e., thermosets) offer unique opportunities to tailor release profiles by simple manipulation of cross-link density, monomer concentration, or monomer molecular weight. Recently, Matras and Chatterjee developed biodegradable cross-linked polyactive esters that challenge the performance of traditional linear polymers by offering greater control over degradation rates, mechanical properties, and release kinetics. Although these cross-linked polyesters showed high biocompatibility and are capable of releasing anticancer, anti-inflammatory, or antimicrobial compounds, the lengthy polymerization times (>1 day) at elevated temperatures (>130 °C) precludes polymerization in the presence of cells, in vivo, or in 3D printing applications. Furthermore, polyesters with sustained degradation typically exhibit localized accumulation of acidic byproducts leading to inflammatory response. Polyactive acetals are promising alternatives as acetals readily hydrolyze under mild aqueous conditions into pH neutral byproducts (alcohols and aldehydes).

Inspired by polyacetal hydrogels, the work herein describes the synthesis of pro-antimicrobial networks via degradable acetals (PANDAs) derived from p-chlorobenzaldehyde (pCB) using thiol–ene photopolymerization, an approach designed to address many of the aforementioned challenges with sequestration of terpene aldehydes. Thiol–ene photopolymerization offers rapid cure kinetics at room temperature, low oxygen inhibition, homogeneous network formation, and high monomer conversion. Thiol–ene, as a step-addition polymerization, ensures that nearly every cross-link junction...
contains a degradable, pCB-derived acetal. Therefore, PANDAs are molecularly designed to undergo complete degradation upon hydrolysis resulting in the release of pCB as an active antimicrobial and antifungal agent, and the generation of inactive low molecular weight degradation byproducts (Scheme 1).

Initially, we synthesized an acyclic diallyl p-chlorobenzaldehyde acetal (pCBA) derived from pCB. For proof-of-concept, pCB was selected from several possible benzaldehyde derivatives with known antimicrobial activity. Benzaldehydes derivatives are known to exert antimicrobial activity through a variety of mechanisms, specifically, inhibition of H^+-ATPase-mediated proton pumping activity, Schiff base/thiazolidine formation with proteins/peptides, and membrane disruption. We synthesized pCBA in acceptable yields via thiazolidine formation with proteins/peptides, and membrane disruption. To facilitate tuning the physical properties of the network, we incorporated the acetal into a hydrophobic polymer, utilizing pentaerythritol tetramercaptopropionate as a photoinitiator. The concentration of TTT can be used to tune the physical properties of the network (i.e., T_g, flexibility, degradation, etc.).

PANDA degradation and subsequent release of pCB were investigated at pH 7.4 and 6.0 by submerging sample disks (25 mm^3) in phosphate buffer solution (PBS) containing octanol to partition the aldehyde. Aliquots from the octanol phase were analyzed via UV–vis spectroscopy to determine the concentration of pCB released over time. Figure 1c shows the pCB release profile at pH 7.4 and 6.0. At physiological pH, a 20 h lag time was observed before pCB was slowly released from the polymer network, with 14% (1.07 mg mL^-1) and 53% (4.19 mg mL^-1) pCB release observed at 24 and 48 h, respectively. Similar trends in degradation and pCB release were observed at pH 6. After 120 h, 72% and 80% pCB release was observed for pH 7.4 and 6.0, respectively. Given that the rate of acetal hydrolysis should be proportional to the H_3O^+ concentration, the rather small increase in rate as a function of pH indicates that incorporation of the acetal into a hydrophobic polymer network drastically decreases the observed rate of hydrolysis. Kim et al. attributed similar trends in degradation of hydrophobic acetal networks to slow diffusion of aqueous buffer into the network.

The photo series in Figure 1d shows the sample disks immersed in PBS buffer solution (pH 7.4) as a function of time.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Cure kinetics, thermomechanical properties, release kinetics, and degradation behavior of PANDAs. (a) Conversion kinetics for 90% pCBA resins cured at 200 mW cm^-2 UV light. (b) Representative thermomechanical plot of the 90% pCBA PANDA. (c) Correlation between pCB released and incubation time at pH 6.0 and 7.4. (d) Time-lapse macroscopic images of degradation of 90% pCBA disks submerged in PBS (pH 7.4) and (e) placed within a 90% humidity chamber under N_2 at 25 °C (Image contrast enhanced for visibility). Error bars indicate the SD (n = 5).
Shortly after immersion, the samples change from transparent to opaque white, but retain their shape with receding dimensions over time. The observed release and degradation behavior are consistent with a surface erosion process (typical of polycetylates), and may be expected given that these materials are relatively hydrophobic (static water contact angle $\approx 90^\circ$). The degradation behavior of these materials becomes exceedingly more interesting when exposed to water vapor (rather than bulk immersion). Figure 1e shows a photo series of PANDA disks subjected to a gentle flow of 90% humidity within a scintillation vial (using N$_2$ as a carrier for water vapor to avoid acidification from CO$_2$). Within 2 h, the PANDA disk hydrolyzed to a point where viscous flow of the sample was observed. At 4 h, the degradation byproducts are shown flowing down the supporting substrate. The rapid progression from an infinite polymer network to viscous flow of low molecular weight degradation products is a consequence of the thiol–ene step polyaddition mechanism, a process that ensures most cross-link junctions contain a degradable linkage and precludes the formation of high MW linear degradation products found in chain growth networks. Notably, these observations point to a rapid bulk degradation process and suggest a vast difference in water vapor permeability (under high humidity) and liquid water permeability (under immersion). While these observations appear to be unique for degradable networks based on current literature, the difference in permeability of water vapor and bulk water is a phenomenon commonly observed in cross-linked poly(dimethylsiloxane). Furthermore, qualitative observations of PANDAs exposed to HCl vapor led to rapid bulk degradation, while exposure to ammonium hydroxide vapor led to retarded degradation (Figure S4). Experiments are underway to quantify the degradation behavior under vapor conditions.

The synthetic design of PANDAs is motivated by the potential use of these materials in antimicrobial/antifungal applications. The antimicrobial activity of PANDAs was initially evaluated via a ZOI assay with clinically isolated strains of E. coli ATCC 43895 [serotype O157:H7], S. aureus RN6390, B. cenocepacia K56–2, and P. aeruginosa PAO1. As shown in Figure 2a, PANDAs containing 0% pCBA showed no ZOI, as expected, and serve as a control for the assay. PANDAs containing >60% pCBA exhibited ZOIs, with larger ZOIs observed for all bacteria with increasing concentration of pCBA (relative to TTT) in the polymer network. The antimicrobial efficacy of the 90% pCBA PANDA material was further investigated using a terminal dilution assay to quantify kill kinetics. Two 25 mm$^3$ 90% pCBA PANDA disks (3.6 mg mL$^{-1}$ pCBA released within 24 h) exhibited more than a 5 log reduction in bacteria count in <12 h against E. coli, S. aureus, and other pathogens (Figure 2b), which translates into kill efficiencies of >99.999%. In interest of developing strategies and materials to mitigate the development of antimicrobial resistance, we evaluated the potential emergence of bacterial resistance of P. aeruginosa against pCBA-based PANDAs using a serial passage mutagenesis assay. For comparison, the same assay was performed using tetracycline. Figure 2c shows the inability of P. aeruginosa to develop resistance toward pCB released from PANDA disks after 20 serial passages as indicated by the absence of an increase in the MIC (25 mm$^3$ disk $\approx 1.3$ mg mL$^{-1}$ pCBA released within 24 h). We note that the concentration of pCB released from the disk after 24 h is comparable to the small molecule MIC at 1.25 mg mL$^{-1}$. In contrast, the MIC value for tetracycline increased after only two passages, with a 10-fold increase in MIC after 20 passages. The results suggest that P. aeruginosa has less propensity to develop resistance against the pCB released from pCBA-based PANDAs, which highlights the potential of PANDAs for use as part of a broader strategy to slow or mitigate the development of antimicrobial resistance.

To further probe the broad-spectrum activity of pCBA PANDAs, we performed zone of inhibition experiments against two opportunistic pathogenic fungi [Candida albicans (C. albicans) and Trihoderma harzianum (T. harzianum)]. Placement of a 25 mm$^3$ 90% pCBA PANDA disk resulted in complete inhibition of both fungi whereas the same size disks containing 0% pCBA exhibited no measurable antimicrobial activity (Figure 2d). Borrowing from the known inhibitory activity of volatile EOs, we exploited the inherent volatility of pCB to inhibit C. albicans, a pathogenic fungus responsible for up to 65% of all candidiasis cases in humans, via a split plate inhibition volatility assay. As shown in Figure 2e, 25 mm$^3$ 90% pCBA PANDA disks were placed on one side of a 9 cm split Petri dish with C. albicans plated on agar on the opposite side. The dish was sealed and incubated for 30 days. In the absence of PANDA disks (control), zero inhibition was observed. Partial inhibition was observed with one disk, while the growth of C. albicans was completely inhibited by two disks for up to 30 days. These experiments exploit the humidity-
triggered degradation and volatility of pCB for noncontact control of fungus. Finally, KB cells (a type of HeLa cells), derived from a glandular cancer of the cervix were used as a model cell line for cytotoxicity. Incubation of the KB cells with a degraded 90% pCBA disk (e.g., media containing PANDA degradation products after 7 days at pH 7.4, equivalent to 2.5 mg mL\(^{-1}\) pCB) and small molecule pCB at the same concentration showed no significant toxicity (>90% viability) to KB cells (Figure 2f).

In summary, we have demonstrated the proof-of-principle for pro-antimicrobial networks via degradable acetals. PANDAs undergo degradation under mild conditions to release pCB, a small molecule active that serves as a potent antimicrobial agent against a broad spectrum of microbes. The humidity triggered degradation and volatile release of pCB was exploited to inhibit the growth of C. albicans without the need for direct contact. Importantly, the broad spectrum antimicrobial efficacy was achieved with minimal cytotoxicity toward KB HeLa cells. Solvent-free, room-temperature, photopolymerization provides a rapid, one-pot approach to cross-linked polyactives with excellent potential for advanced processing techniques, including 3D printing. The PANDA approach presented here is readily applicable to other bioactive aldehydes pointing to the potential use of these materials within pharmaceutical, biomedical, and agricultural industries.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.7b00009.

Experimental details for the synthesis and characterization of pCBA, PANDAs, antifungal/antibacterial assays, and cytotoxicity toward KB cells (PDF).

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**Notes**

The authors declare no competing financial interest.

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