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Review

Photoactivated Chromophore for Keratitis-Corneal Cross-linking (PACK-CXL)—A Scoping Review Based on Preclinical Studies

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Purpose: Photoactivated chromophore for keratitis-corneal cross-linking (PACK-CXL) stabilizes the corneal stroma and eliminates microorganisms. Numerous PACK-CXL protocols, using different energy sources and chromophores, have been applied in preclinical studies, including live animal studies, with various experimental designs and endpoints. So far, a systematic mapping of the applied protocols and consistency across studies seems lacking but is essential to guide future research.

Methods: The scoping review protocol was in line with the *JBI Manual for Evidence Synthesis*. Electronic databases were searched (Embase, MEDLINE, Scopus, Web of Science) to identify eligible records, followed by a two-step selection process (title and abstract screening, full text screening) for record inclusion. We extracted information on (1) different PACK-CXL protocol characteristics; (2) infectious pathogens tested; (3) study designs and experimental settings; and (4) endpoints used to determine antimicrobial and tissue stabilizing effects. The information was charted in frequency maps.

Results: The searches yielded 3654 unique records, 233 of which met the inclusion criteria. With 103 heterogeneous endpoints, the researchers investigated a wide range of PACK-CXL protocols. The tested microorganisms reflected pathogens commonly associated with infectious keratitis. Bacterial solutions and infectious keratitis rabbit models were the most widely used models to study the antimicrobial effects of PACK-CXL.

Conclusions: If preclinical PACK-CXL studies are to guide future translational research, further cross-disciplinary efforts are needed to establish, promote, and facilitate acceptance of common endpoints relevant to PACK-CXL.

Translational Relevance: Systematic mapping of PACK-CXL protocols in preclinical studies guides future translational research.

Background

Infectious keratitis is an inflammatory disease of the cornea, which threatens vision and requires immediate treatment. Various microorganisms are involved in the pathology of infectious keratitis, such as bacteria, fungi, amoebas, and viruses. The inflammatory response to an infection activates proteolytic enzymes that destroy collagen in the corneal stroma, which increases lesion depth and size.[1–5] Therefore the rapid initiation of a treatment that stops corneal tissue destruction and eliminates pathogens is essential for treatment success.

Predominant causes of infectious keratitis are often host-specific and, for both humans and animals, depend on geographic location and exposure to risk factors. Fungal keratitis is most prevalent in tropical and subtropical climates[6] and may harm more than a million people annually.[7] Bacterial infections are more prevalent in established economies,[8,9] where they lead to an incidence of six to 40 cases per 100,000 people/year.[6,10]

Studies in companion animals revealed that dogs and horses suffer from both bacterial and fungal keratitis, with a reported prevalence of 0.8% for infectious keratitis in dogs.[4,11–16] This number will likely increase in the future because of the increasing popularity of

brachycephalic (short-nosed) dog breeds with compromised ocular anatomy. Brachycephalic dogs have an odds ratio of 6 for developing infectious keratitis, compared to typical mesocephalic or dolichocephalic dogs.[11,17]

Drug resistance among pathogens threatens infectious keratitis treatment success in humans and animals. The World Health Organization has declared antimicrobial resistance one of the major public health threats of the twenty-first century.[18] Because rapid treatment initiation before culture and sensitivity test result availability is crucial for treatment success, the selected antibiotics typically have a broad spectrum to cover the most likely bacterial pathogens while considering existing antibiotic resistances.[19] Therefore fluoroquinolones are often the basis of first-line treatment while awaiting culture and sensitivity test results. Unfortunately, resistance to fluoroquinolones is increasingly common among ocular bacterial strains.[10,20–22]

Corneal cross-linking (CXL) was first introduced as a treatment for keratoconus (progressive thinning of the cornea), with a specific CXL setting known as the Dresden protocol. In this protocol, a 0.1% riboflavin (chromophore) solution is first applied to the de-epithelialized cornea for 30 minutes. Second, the cornea is exposed to ultraviolet A irradiation (energy source) for 30 minutes at 3 mW, delivering an energy dose (fluence) of 5.4 J/cm^2. CXL gained interest as a treatment for infectious keratitis because of two properties: its stabilizing effect on the corneal stroma (mostly by improvement of corneal stromal resistance to enzymatic digestion) and the elimination of pathogens.[23]

CXL is a potential treatment alternative for infectious keratitis in human and veterinary patients with a mechanism of action independent from antibiotics. In 2008, the routine Dresden CXL protocol was effectively tested for the first time in humans with infectious keratitis.[24] Initially, only infectious keratitis patients refractory to medical therapy were treated. In 2013, the name Photoactivated Chromophore for Keratitis-Corneal Cross-linking (PACK-CXL) was adopted at the 9th CXL Congress in Dublin. This name change was implemented to distinguish the use of CXL for the treatment of keratoconus from the use of PACK-CXL for the treatment of infectious keratitis and to make room for the use of other, potentially more efficient, chromophores and energy sources.[25] In 2014, the first studies that described the use of PACK-CXL in companion animals were published.[26–28]

Clinically used PACK-CXL protocol settings are being adjusted, because the Dresden CXL protocol may be insufficient for the treatment of infectious keratitis.[25] A number of systematic reviews that summarize completed clinical trials on PACK-CXL efficiency have been published.[29–32] These reviews highlight the dominance of the Dresden protocol. However, a tendency towards treatment in early disease stages and a preference towards the use of accelerated, high fluence protocols is noticeable.[33–39]

PACK-CXL is intensively tested in in vitro and in vivo laboratory and clinical animal studies, to define the best PACK-CXL settings against various pathogens and at different infectious keratitis stages. So far, a systematic mapping of the PACK-CXL protocols applied in preclinical studies that could guide future translational research is lacking. The aim of this scoping review is to comprehensively map preclinical PACK-CXL studies to identify explored protocols and pathogens and the methods and endpoints used to determine the antimicrobial and tissue stabilizing effects of PACK-CXL.

Methods

Registration

The full study protocol is available at Open Science Framework at www.osf.io/ypxjs/.

Protocol Design

The scoping review protocol was drafted in line with the JBI Manual for Evidence Synthesis.[40] It was reported according to PRISMA-ScR[41] (checklist available at www.osf.io/ypxjs/). The framework consists of five stages: (1) identification of the research questions and objectives; (2) identification of relevant studies; (3) selection of studies; (4) extraction and charting of data; (5) collation, summation, and reporting of results. A complete review protocol was released in advance: www.osf.io/ypxjs/.

Identification of the Research Questions and Objectives

This review aims to comprehensively map information available in the existing literature on PACK-CXL preclinical studies, which includes in vitro studies and in vivo laboratory and clinical animal studies. To meet the research objectives, the following research questions (RQ) were addressed:

RQ1: What PACK-CXL protocol modifications have been investigated? Modifications in the following protocol elements were considered: chromophore type, concentration and carrier, energy source, wavelength, energy intensity level and delivery time, and fluence (the total amount of energy delivered).

RQ2: Which pathogens were tested?
RQ3: Which types of study design and experimental setting were used in in vitro studies and in preclinical animal studies?
RQ4: Which endpoints were used to assess PACK-CXL-relevant treatment effects?

Identification of Relevant Studies

First, a literature search limited to one online database (Google Scholar) was performed to gain knowledge regarding relevant search terms. After analyzing the index terms used to describe the retrieved articles and the words used in the article titles and abstract texts, keywords to be used in the final literature search were identified. An experienced librarian then established the final search strategy and performed the literature search across five databases: Embase, Cochrane, MEDLINE, Scopus, and Web of Science. The full electronic search strategy for all databases is available at the online repository www.osf.io/ypxjs/. The initial search was performed in December 2020 and updated in December 2023.

Selection of Studies

Only peer-reviewed primary research publications and published conference abstracts from scientific ophthalmology or vision science meetings were eligible for inclusion into the scoping review. The described work needed to involve the use of animals with spontaneous or experimentally induced infectious keratitis, or be laboratory-based work using bacteria, fungi, or amoebas. Furthermore, the records needed to include at least one endpoint relevant to antimicrobial or tissue-stabilizing effects of PACK-CXL. Language restrictions were not imposed. Records based on non-photoactivated CXL and records published before the year 2000 were excluded from the review. The second exclusion criterium was chosen because CXL had not been investigated as a treatment for infectious keratitis before the year 2000. Endpoints related to the biomechanical effects of CXL that were investigated in records included in the review were not considered because such endpoints are mainly relevant for the treatment of keratoconus and not infectious keratitis.

Two reviewers screened the records' title and abstract list and applied the eligibility criteria in parallel. Three questions were asked to establish record eligibility, and records were included if the answer to all three questions was "yes."

- Does this study involve the use of an intervention or treatment method based on the combination of a chromophore (photosensitive agent) and energy source?
- Are any of the study findings relevant to PACK-CXL treatment efficacy in terms of antimicrobial activity or tissue stabilization (tissue resistance to enzymatic digestion, structural changes, or treatment depth)?
- Can this study be considered preclinical (in vitro or in vivo clinical or laboratory-based animal study)?

In case of disagreement, a third reviewer was included in the decision-making process and a consensus was sought. EndNote software[42] was used to generate publication lists and a Google Sheets document was used to store the decisions regarding record inclusion or exclusion. The reliability of agreement

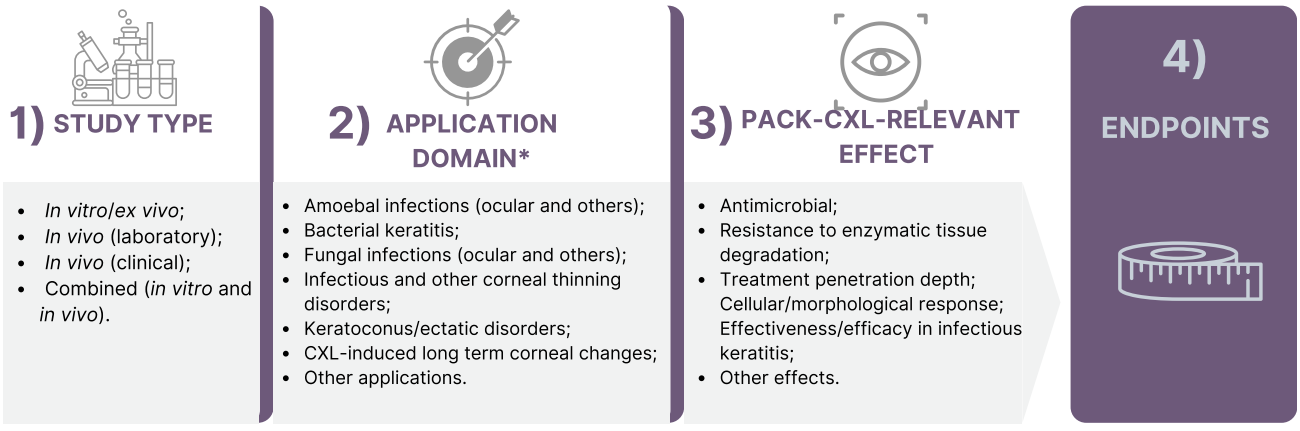


Figure 1. Data extraction and classification process. The process contained four stages: 1. Record classification into one of four study types; 2. Record classification into one of seven application domains; 3. Record classification into one of six PACK-CXL-relevant effect categories. Here, more than one effect category was possible per record and application domain; 4. Grouping of recorded endpoints used to measure PACK-CXL-relevant effects under new common names, and according to study type, application domain and PACK-CXL-relevant effect category.

among the three reviewers was checked with Fleiss kappa.

Extraction and Charting of Data

In scoping reviews, the data extraction process is referred to as "data charting." First, animal reporting guidelines (the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research[43] and ARRIVE guidelines[44]) were searched to identify items of relevance for this scoping review. Extracted items were then collected in seven blocks: (1) publication-related information (year, language, open source, preregistration, funding source), (2) research question, (3) PACK-CXL protocol characteristics, (4) pathogens, (5) study design/experimental settings, (6) animal model, and (7) measured endpoints. The data extraction forms are provided in the project protocol (www.osf.io/ypxjs/). Charting of the data was performed in Covidence software.[45]

One reviewer extracted data from all eligible records. In case of doubt, the reviewer discussed items with a second reviewer to reach a consensus. Data from 10% of the records was extracted in parallel by another reviewer, to test the extraction forms.

Collation, Summation, and Reporting of Results

All results were presented separately for the four study types encountered: in vitro/ex vivo (laboratory based), in vivo (animal laboratory experiments),

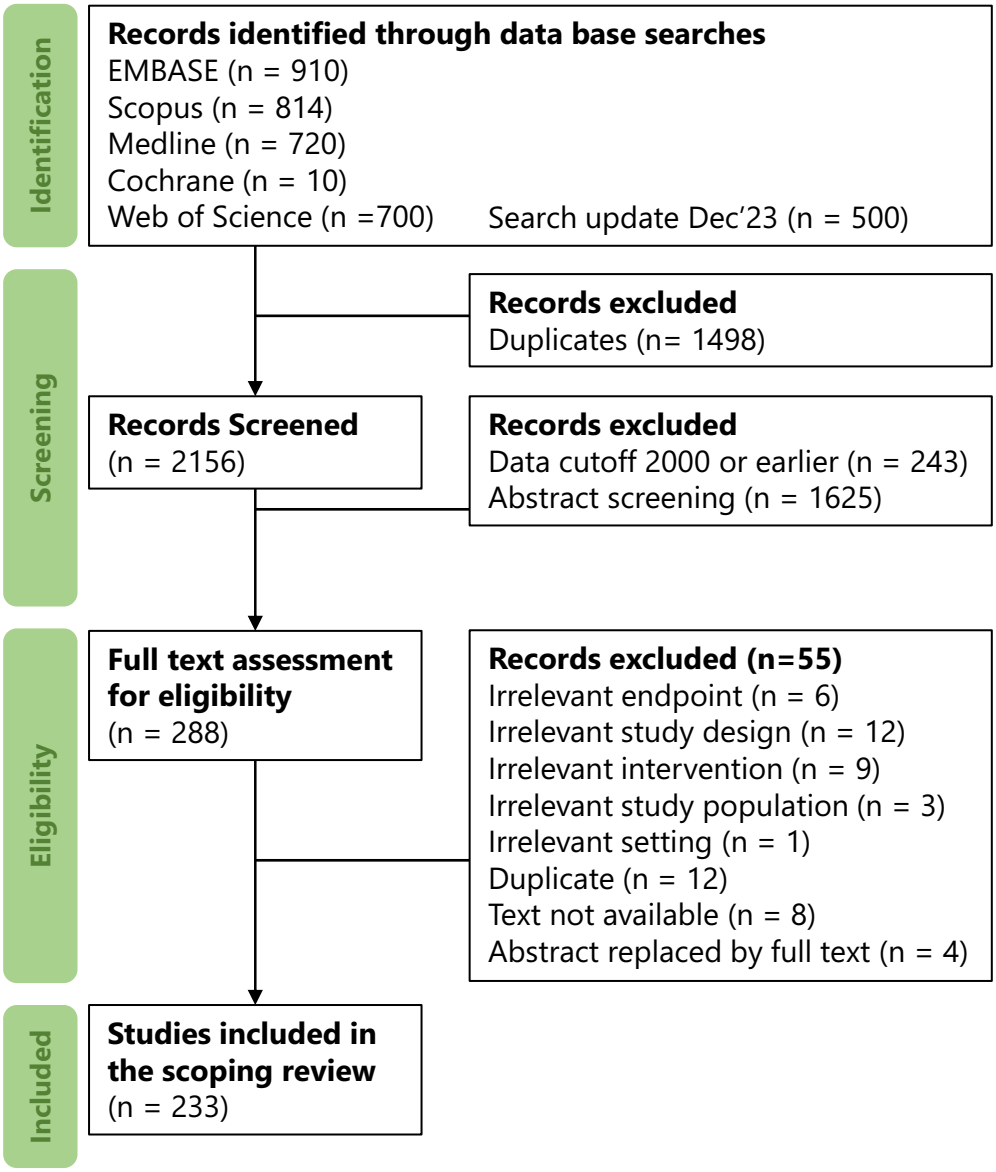


Figure 2. PRISMA flow diagram of record eligibility screening.

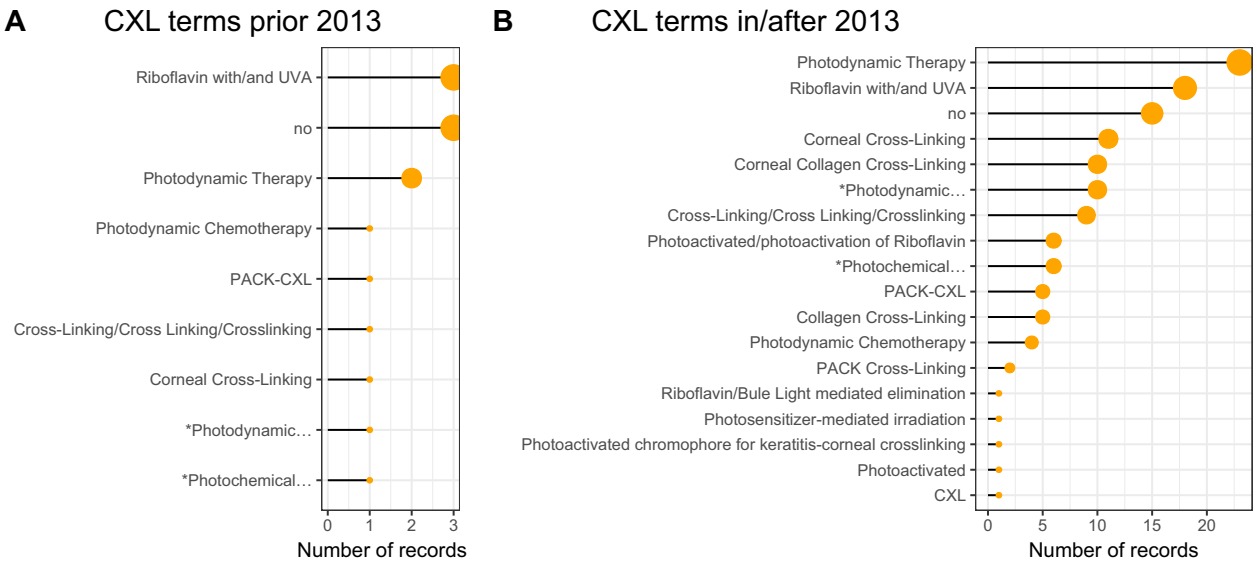


Figure 3. Original terms used in record titles to describe investigated interventions. The original terms describing the investigated interventions were extracted from the titles of records that investigated antimicrobial CXL efficacy or CXL effectiveness/efficacy against infectious keratitis. (**A**) terms in records published before 2013. (**B**) Terms in records published in or after the year 2013. "No": indicates that the intervention was not named in the record title. *Photodynamic = Photodynamic antimicrobial/inactivation/elimination/eradication; *Photochemical = Photochemical activation/eradication/therapy/cross-linking.

in vivo (animal clinical studies), and records that combined both in vitro and in vivo methodology in one publication (Fig. 1). A PRISMA flow diagram of record eligibility screening is supplied in Figure 2. Further publication-relevant information is presented in Supplementary Figure S1. The full list of included records is available on our project repository (www.osf.io/ypxjs/) and as Supplementary Table S1. An attempt was made to assess whether the PACK-CXL nomenclature was harmonized in the field since 2013, the year in which the name PACK-CXL was adopted by opinion leaders in the field.[25] The frequency was mapped with which the term PACK-CXL, or an equivalent term indicating a photochemical intervention or treatment method, was used in article titles released before and since 2013 (Fig. 3). Information collected under research questions RQ 1 through 3 is summarized in Tables 1 through 3 and in Figure 4.

RQ4: Endpoints Used To Assess PACK-CXL Treatment Effects

Special emphasis was placed on mapping the recorded endpoints that were used to assess PACK-CXL-relevant treatment effects in the studies. To make mapping possible, domains of specific (PACK-)CXL study applications were created, based on the study purpose as described by the article or conference abstract authors. The following domains were created: amoebal infections (ocular and others), bacterial keratitis, fungal infections (ocular and others), infectious and other corneal thinning disorders, keratoconus/ectatic disorders, CXL-induced long-term corneal changes, other applications (Boston keratoprosthesis, bullous keratopathy, and sterile melting/keratoconus). The endpoints were systematically mapped through frequency maps, which were stratified by study type (in vitro/ex vivo; in vivo [laboratory]; in vivo [clinical]; combined [in vitro and in vivo]) and study application domain. Figure 1 illustrates this process. Figure 5 and Supplementary Figures S2 and S3 illustrate the results.

It is important to note that this approach describes the popularity of the used endpoints rather than the strength of evidence. The latter would be more appropriately described by measures of effect size accompanied by assessment of bias. However, because of inconsistent reporting and interpretation of endpoints, reliable mapping of effect sizes was not feasible.

For ease of interpretation, endpoints were organized into six categories representing the investigated PACK-CXL-relevant effects (Fig. 1): antimicrobial, resistance to enzymatic tissue degradation, treatment penetration depth, cellular/morphological response, effectiveness/efficacy in infectious keratitis, and other effects. Each record was classified into only one study type and one application domain, but more than one PACK-CXL–relevant effect could be investigated within the same application domain.

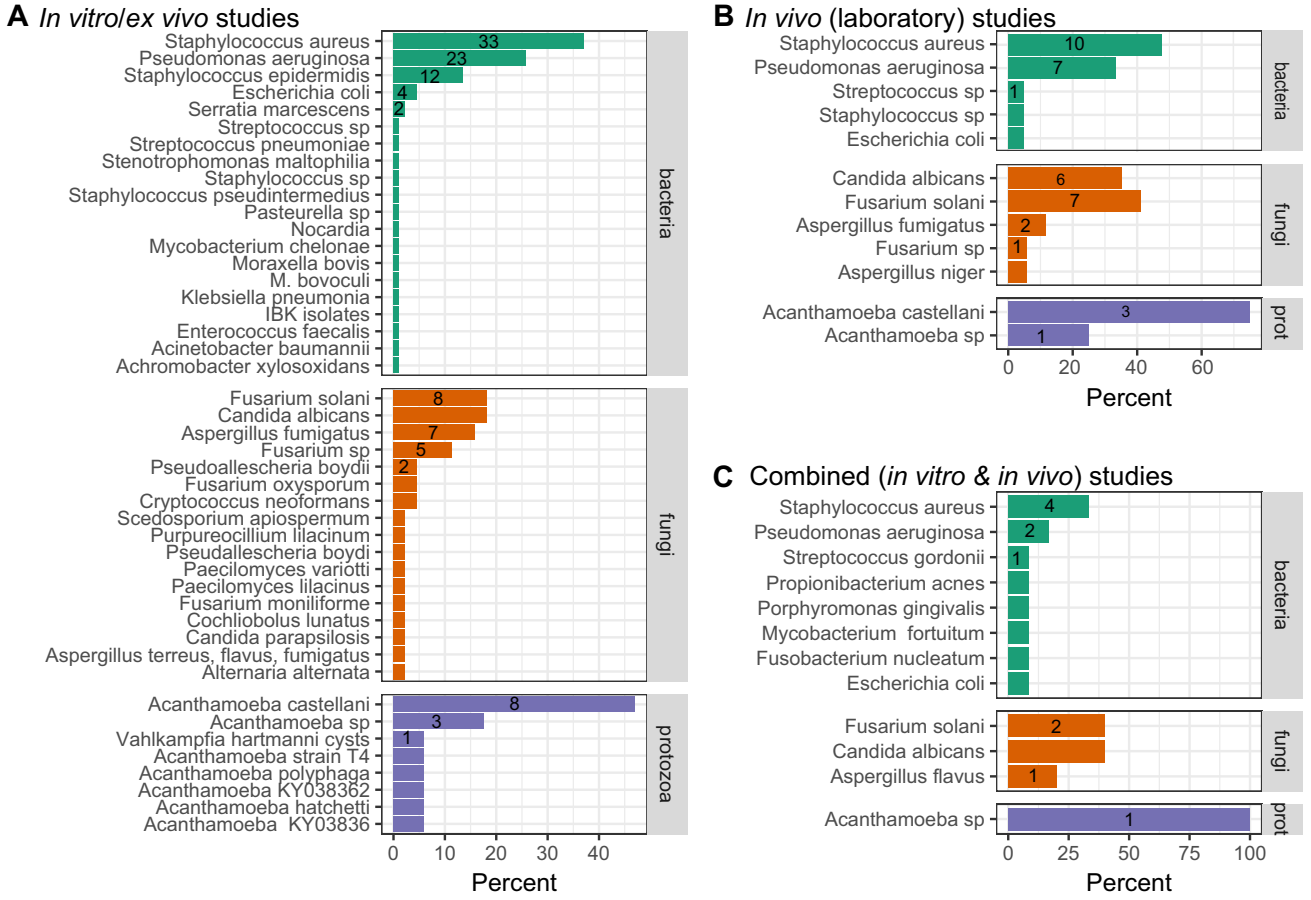


Figure 4. Pathogens tested in PACK-CXL studies (RQ 2). The pathogen species used to investigate PACK-CXL-relevant effects in three study types are presented: (**A**) in vitro/ex vivo; (**B**) in vivo (laboratory); and (**C**) studies combining both in vitro and in vivo models. Studies including clinical animal patients were excluded from the graph, since pathogen detection largely depends on the culture methodology in such studies. Studies involving bacteria, fungi and protozoa are presented in green, orange, and purple, respectively. Prot, protozoa.

We then grouped the recorded endpoints according to study type, application domain and PACK-CXL-relevant effect category, giving them new common names, based on the measurement method and SI units used (example of a new common name: "bacterial elimination [CFU or CFU/ml]" in Fig. 5C). All new "common" names together with their definitions and examples of original endpoint terms extracted from the eligible records, are available in the online repository (www.osf.io/ypxjs/).

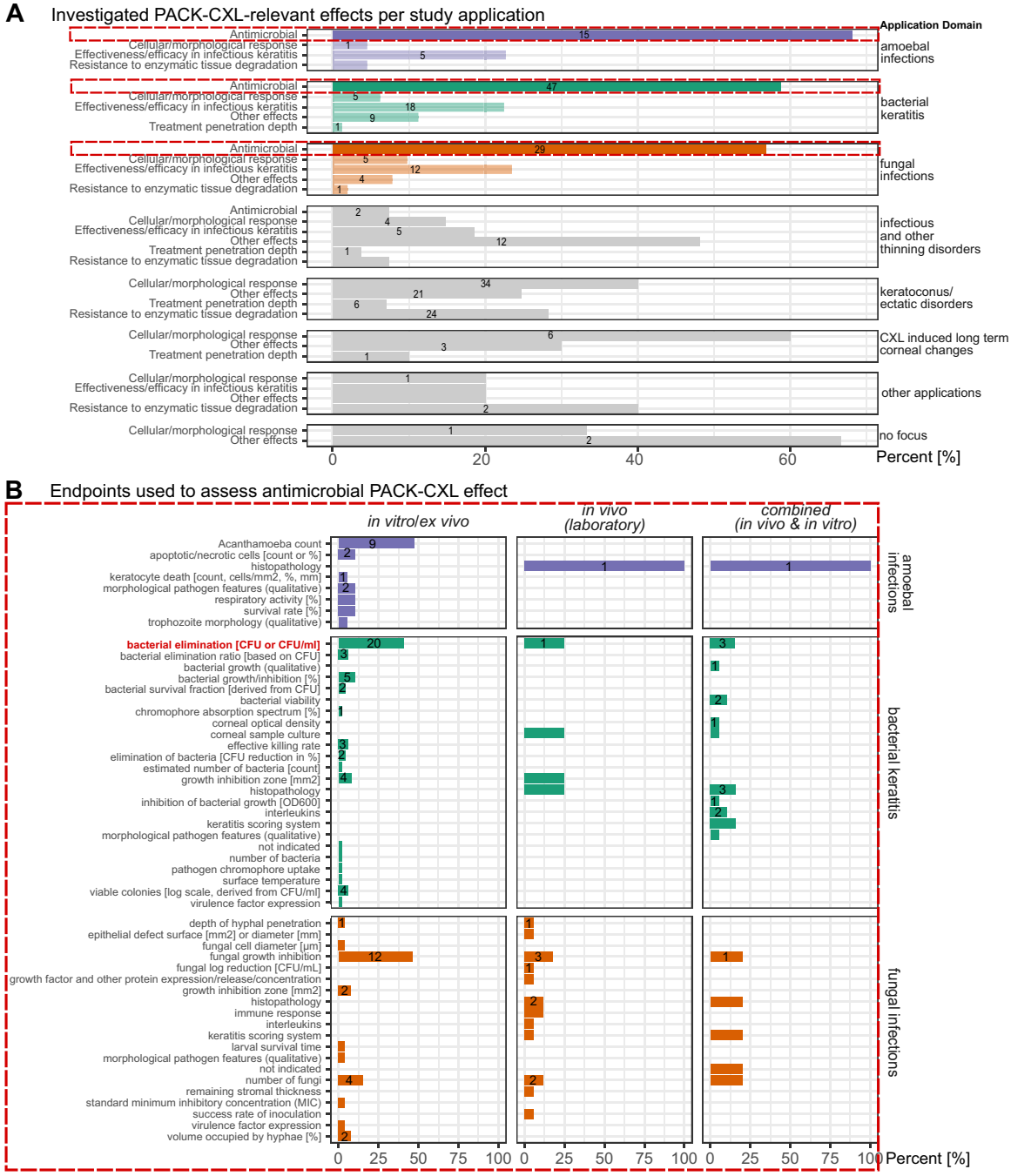
Deviations From the Original Protocol

The results of the literature search in the electronic databases were considered sufficient, and 68 abstracts were identified. As a result of the large number of abstracts identified in the literature search, we decided to not manually scan the abstracts from the following conferences, as was stated in the prereleased complete study protocol available online (www.osf.io/ypxjs/): CXL Experts' Meeting, European Society of Cataract and Refractive Surgeons (ESCRS), Association for Research in Vision and Ophthalmology (ARVO), American Academy of Ophthalmology (AAO), European College of Veterinary Ophthalmologists (ECVO). The authors consider this decision justified because published articles have undergone peer review and many abstracts might later have been published as peer-reviewed publications.

Results

Characteristics of Included Studies

Our search yielded 3654 unique records, 288 of which were deemed eligible for full-text review. Of these, 233 records were eligible for inclusion (in vitro/ex



C Original terms used to identify "**bacterial elimination [CFU or CFU/ml]**"

Common name	Original terms
bacterial elimination [CFU or CFU/ml]	bacterial elimination [CFU], bacterial growth [CFU], antimicrobial effect [CFU], number of colony-forming units, mean corneal [CFU], efficacy [CFU], % reduction [CFU/ml], microbiological analysis [CFU], bacterial viability [CFU], antibacterial efficacy [CFU], elimination of bacteria [CFU], cell viability [CFU/ml], cell survival [CFU/ml], bacteria concentration [CFU/ml], bacteria reduction [CFU], bacterial load [CFU], bacterial growth inhibition [CFU], bacterial load [CFU], the growth activity [CFU], colonies numbers per agar plate, counting surviving colonies in the irradiation zone

Figure 5. Endpoints used to assess PACK-CXL-relevant effects (RQ 4): general and antimicrobial effect-specific overview. (**A**) Bar plots representing the frequency of use of the various endpoint categories per PACK-CXL application domain. The domain "Other applications" includes bullous keratopathy, Boston keratoplasty, and sterile melting/keratoconus. The domains "amoebal infections" and "fungal infections" included isolates from ocular (infectious keratitis) and/or non-ocular infections. The endpoints used for the assessment of the antimicrobial effects of PACK-CXL are indicated with an interrupted red rectangle and are presented in more detail in Figure 5B. (**B**) Overview of endpoints (common names under which the original endpoint term definitions that were extracted from the eligible records were grouped) →

←
used to measure the antimicrobial effects of PACK-CXL in studies assigned to the amoebal, bacterial, and fungal infection domains. (**C**) The terminology used in the original records to describe the endpoints grouped under the same common endpoint name "bacterial elimination [CFU or CFU/mL]."

vivo: n = 137; in vivo [laboratory]: n = 72; in vivo [clinical]: n = 9; combined [in vitro and in vivo]: n = 15), composed of 68 conference abstracts and 165 full text publications (Fig. 2, Supplementary Fig. S1). The list of included records is available on our project repository (www.osf.io/ypxjs/) and as Supplementary Table S1. The number of included records published per year and publication-relevant information such as: language, origin of the research collaboration and financial support are presented per study type as Supplementary Figure S1.

The initial agreement between reviewers at the abstract eligibility screening stage was poor (three raters, Fleiss' $\kappa = 0.20$), but a consensus was reached through discussions. Sixty-two records with a main research focus on keratoconus/ectatic disorders were included, because the answer to all three eligibility criteria-related questions was "yes" (Methods section: Selection of studies), and the investigated CXL effects (cellular/morphological changes; resistance to enzymatic tissue degradation; treatment penetration depth; other effects) were therefore deemed relevant for infectious keratitis treatment.

Figure 3 illustrates that a unified nomenclature to identify the use of CXL for the treatment of infectious keratitis has not been adopted across the field, either before or since 2013. Across records, the most popular terms included "Photodynamic Therapy" (n = 23), and phrases containing the words "Riboflavin with/and UVA" (n = 18), and "Corneal Cross-Linking" (n = 11). "Photoactivated" or "PACK-CXL" were used in the titles of 15 records.

RQ1: What PACK-CXL Protocol Modifications Have Been Investigated?

Nineteen distinct chromophores were investigated in the in vitro/ex vivo studies. This number was decreased to seven distinct chromophores that were investigated in the in vivo studies (Table 1). Riboflavin was the only chromophore used in clinical animal studies. Riboflavin received the greatest attention across all study types, with varying riboflavin concentrations, additives, and CXL fluences, and irradiation intensities investigated. Rose Bengal was the second most popular chromophore. Information regarding PACK-CXL protocol parameters was missing in five studies and was incomplete in many other records. For example, fluence was frequently expressed as total energy level without specified irradiation intensity or vice versa. We have not presented information regarding chromophore replacement during the irradiation phase because this information was missing in most studies. Various SI units were used in the context of chromophore concentration, total fluence, and irradiation intensities.

RQ2: Which Pathogens Were Tested?

Pathogens were used in a total of 133 records (42 abstracts and 91 full texts), with bacteria being the most commonly used (n = 69). The majority of the pathogen strains that were used originated from clinical cases of keratitis (Table 2). Also, the pathogen species that were most commonly used correspond to the pathogens that are most frequently encountered in clinical patients (Fig. 4).[12–14,16,46–48] Unfortunately, information on pathogen origin, concentration/load, or antimicrobial resistance was missing from many, even full text, records. For example, pathogen origin was not listed in 25 of 91 full text records (in vitro/ex vivo [n = 6/53], in vivo laboratory [n = 12/26], combined [in vitro and in vivo] studies [n = 7/12]), and information specifying pathogen concentration/load was missing from 16 of 91 full text records (in vitro/ex vivo [n = 10/53], in vivo laboratory [n = 4/26], combined [in vitro and in vivo] studies [n = 2/12]). Furthermore, information regarding antimicrobial resistance was missing from 55 of 78 full text records using wild-type pathogens. Finally, a variety of units was used to specify pathogen concentration (e.g., McFarland standard [McF], colony forming units [CFU], CFU/mL, CFU/0.1 mL, CFU/50 mL, cells/mL).

RQ3: Which Types of Study Design and Experimental Setting Were Used?

A wide range of experimental models was used to investigate PACK-CXL-relevant effects. The most commonly used experimental model in the in vitro/ex vivo studies was a PACK-CXL irradiated pathogen suspension (Table 3). Here, the suspensions were placed in plates with different well sizes (12-, 24-, or 96-well plates), with some authors providing additional information and recording the suspension column height (200–400 µm). Further in vitro/ex vivo study models ranged from cell culture to culturing of whole globes.

Table 1. Investigated PACK-CXL Protocol Modifications (RQ 1)

Chromophore Type	N	Concentration	Carrier	Combined With	Energy Type	Wavelength (nm)	Total Fluence (J/cm^2)	Irradiation Intensity (mW/cm^2)	Irradiation Mode	Record ID
In vitro/ex vivo studies										
Riboflavin	92	0.001, 0.002, 0.01, 0.05, 0.1, 0.14, 0.2, 0.25, 0.3, 2.5%; 100, 200, 300, 400 µM	Dextran 20% 25%, disodium hydrogen phosphate, distilled water, NaCl, HMPC and 0.01% BAC	H_2O_2 0.004%, 30%; 0.5 mM Iron; fluorescein (10-0.1%); 0.001% BAC; Amphotericin B; 4% açai (Euterpe oleracea); sodium persulfate; iontophoresis*	UVA, LED light, blue light	365, 370, 375, 412	2, 2.4, 3, 4, 5.375, 5.4, 6.75, 7.2, 8, 10.8, 28.4, 32.4, 48.6, 64.8	1, 2, 2.8, 2.9, 3, 5.3, 6, 6.4, 7.3, 8, 9, 18, 28.6, 30, 36	Continuous; pulsed	A
Rose Bengal	23	0.001%, 0.03%, 0.1%, 0.2%	Disodium, distilled water, NaCl	H_2O_2; iontophoresis	Green light, LED light	518, 520, 532	100, 150	0.25 (W/cm^2)	Continuous	B
Chlorin/Ce6/porphyrin derivative/TONS 504	17	0.1, 1, 10, 20 mg/L; 10 µg/mL; 7.5 to 60 mM; 20, 100 nM	None	EDTA	LED light red light	405, 660, 670	1.86, 9.3, 10, 18, 18.6, 20, 24, 30, 60	35, 31	Continuous	C
Methylene blue/New methylene blue N	5	No info.	None	None	No info.	No info.	No info.	9.8	Continuous	D
Fluorescein	2	0.01%, 0.1%, 0.2%, 1%, 10%	None	None	UVA	365, 370	No info.	No info.		145, 70
Verteporfin	2	6 mg/mL	None	None	Non-thermal laser	689	No info.	600	Continuous	218, 210
[Ru(bpy)3]2+	1	0.1, 1 mM	No info	SPS	Light	430	(40 min)	3	Continuous	337
Tetra-cationic porphyrins (H2TMeP, ZnTMeP)	1	40 µM	No info.	No info.	LED light	no info.	45	25	Continuous	354
Toluidine blue	1	20 µM	None	None	LED light	625	13.14	0.68, 1.07, 2.47, 5.27, 7.3	Continuous	51
Titanium dioxide	1	0.8 mg/mL	None	Chlorhexidine (0.2 mg/mL)	UVA	365	No info.	2.2	Continuous	149
Polyethylene glycol	1	10%	None	None	UVA	No info.	No info.	No info.	Continuous	188
Pentacyclic S137	1	no info.	None	None	No info.	635	15	No info.	Continuous	80
Indocyanine green (ICG)	1	12.5, 25, 50, 100 mg/mL	None	None	Near infrared light		50, 100, 200	65.5	Continuous	14
Hypocrellins B	1	0.0625, 0.125, 0.25, 0.5, 1 mg/mL	None	None	Sodium lamp	>470	90	50	Continuous	182
Curcuminoids salt	1	500, 1000, 1500 µg/mL	None	None	No info.	460	30, 50	36	Continuous	175
Bperox (riboflavin + hydrogen peroxide)	1	0.1%-0.004%	None	None	UVA	365	5.4	3, 10	Continuous	66
Aluminum phthalocyanine tetrasulfonate (AlPCS4)	1	0.2, 0.4, 1, 2, 3 µM	None	None	Red light	630-700	No info.	50, 100	Continuous	120
No information	3									
In vivo (laboratory) studies										
Riboflavin	54	0.1%, 0.2%, 0.22%; 1 g/L	Dextran 20%	HPMC 1.0%; EDTA 0.1%, Trometanol 0.05%; 1% voriconazole; tobramycin; iontophoresis†	UVA no info.	360, 365, 370, 440	2.16, 3.24, 5.4, 7.2	1.8, 2, 3, 5, 9, 9.7, 18, 45, 90	Continuous; pulsed	E
Rose Bengal	10	0.05%, 0.1%	PBS, BSS, distilled water	Polypyrrole-coated gold nanoparticles (AuPpy NP)	Green light	525, 532	5.4, 100, 150	6; 0.2, 0.25 (W/cm2)	Continuous	F

Table 1. Continued

Chromophore Type	N	Concentration	Carrier	Combined With	Energy Type	Wavelength (nm)	Total Fluence (J/cm^2)	Irradiation Intensity (mW/cm^2)	Irradiation Mode	Record ID
Chlorin/Ce6/porphyrin derivative/TONS 504	5	0.01%, 0.05%, 0.1%; 1 mg/mL	PBS	None	Red light LED light	660, 670	24, 30, 60	no info.	Continuous; pulsed	G
WST-D	1	no info.	Dextran	None	Near-infrared light	755	No info.	No info.	Continuous	141
Toluidine blue	1	1 nM	None	None	Red light	630	No info.	87.8	Continuous	35
Quercetin	1	No info.	No info.	No info.	No info.	No info.	No info.	No info.		119
No information	4									
In vivo (animal clinical) studies										
Riboflavin	9	0.10%	Dextran 20%	None	UVA	365, 370	5.4	3, 30	Continuous	H
Combined (in vitro and in vivo) studies										
Riboflavin	4	0.10%	Dextran 20%	None	UVA	365	5.4	3, 45	Continuous	124, 121, 2, 304
Rose bengal	2	0.1%, 2 µM								332, 358
AIEgens (IQ-TPA)	1	1, 2 µM	PBS	None	Light	No info.	No info.	20		332
TONS 504	1	5 µg/mL								363
Octacationic phthalocyanine	1	1 µM	None	None	No info.	675	No info.	No info.	Continuous	90
Methylene blue	1	0.5% (in vivo); various (in vitro)	None	None	Halogen lamp	560 to 780	97.5 (in vivo); 100, 150, 200 (in vitro)	100	Continuous	50
Hematoporphyrin	1	3 mg/mL (in vivo); 0.5, 1, 2, 3 mg/mL (in vitro)	None	None	LED light	630	9 (in vivo); 0.9-9 (in vitro)	No info.	Continuous	164
p(GM-r-FM)-I	1	125 µg/L	No info.	No info.	No info.	532	(10 min)	433	Continuous	347
PFH/F-I	1	500 µg/mL	No info.	No info.	No info.	532	(10 min)	50	Continuous	360
TTVP	1	No info.	No info.	No info.	UV	365	(15 min)	20	Continuous	362
Upconversion nanoparticles (UCNP)	1	500 µg/mL	No info.	SiO2-AIE, SiO2-NO	Laser	808	(30 min)	0.4 (W/cm2)	Continuous	364
No information	1									

N, Sum of manuscripts that investigated the listed chromophore type; no info., no information; BAC, benzalkonium chloride; EDTA, sodium ethylenediaminetetraacetic acid; HPMC, hydroxypropylmethylcellulose.

*Or prolonged iontophoresis, with penetration enhancers such as: gum cellulose 0.44% sodium chloride, and 0.01% BAC; Tris-aminomethane 0.05%, EDTA 0.1%, NaHPO42H2O 0.21%, and NAH2PO42H2O 0.36%; TRIS-hydroxymethyl aminomethane and EDTA; sodium phosphate monobasic dihydrate, and sodium chloride

†0.02 or 0.04% BAC; 1.0% HPMC; 0.02% BAC, 0.01% EDTA, and 0.5% carboxymethylcellulose; continuous irradiation was assumed if no information about pulsed irradiation was found.

Records can be found at the supplementary materials in Supplementary Table S1 under the following ID numbers: A: 218, 217, 216, 215, 214, 213, 212, 211, 210, 209, 208, 204, 201, 199, 197, 194, 189, 172, 171, 168, 166, 165, 162, 155, 146, 145, 144, 140, 127, 125, 123, 122, 116, 110, 109, 108, 107, 105, 104, 103, 102, 101, 100, 96, 95, 94, 87, 86, 85, 75, 71, 70, 67, 65, 64, 63, 61, 59, 58, 57, 56, 53, 52, 47, 46, 43, 37, 30, 28, 27, 24, 23, 1, 200, 191, 93, 310, 311, 317, 318, 319, 320, 324, 327, 337, 338, 343, 345, 346, 348, 350, 366; B: 208, 206, 204, 167, 166, 165, 162, 144, 114, 104, 88, 76, 71, 22, 320, 325, 333, 341, 348, 353, 355, 356, 367; C: 177, 106, 79, 78, 54, 41, 40, 38, 33, 32, 31, 19, 181, 180, 48, 326, 331; D: 80, 91, 331, 346, 357; E: 205, 198, 196, 194, 192, 185, 184, 174, 163, 154, 152, 143, 141, 136, 134, 133, 132, 129, 128, 119, 118, 117, 113, 112, 111, 82, 60, 36, 34, 29, 20, 17, 16, 15, 12, 11, 10, 9, 8, 4, 306, 307, 308, 312, 314, 315, 321, 322, 323, 329, 344, 352, 359, 361; F: 203, 156, 115, 97, 89, 21, 6, 330, 351, 365; G: 169, 77, 13, 179, 303; H: 161, 160, 138, 137, 74, 45, 301, 305, 309.

Table 2. Origin of Pathogens Used in PACK-CXL Studies (RQ 2)

Pathogen Kingdom	Pathogen Origin	n	N	Record ID
In vitro/ex vivo studies				
Bacteria	Clinical (not ocular)	4	46	63, 46, 19, 14
	Keratitis case	19		A
	Laboratory	8		B
	No info.	15		—
Fungi	Clinical (not ocular)	1	20	346
	Keratitis case	9		C
	Laboratory	4		30
	No info.	6		197, 47, 180
Protozoa	Clinical and environmental	1	14	85
	Keratitis case	6		D
	Laboratory	4		102, 78, 27, 326
	No info.	3		
In vivo (laboratory) studies				
Bacteria	Clinical (not ocular)	1	15	13
	Keratitis case	2		35, 308
	Laboratory	3		29, 20, 321
	No info.	9		
Fungi	Keratitis case	4	13	154, 128, 306, 307
	Laboratory	2		312, 365
	No info.	7		—
Protozoa	Clinical (not ocular)	1	4	194
	Keratitis case	1		203
	Laboratory	1		303
	No info.	1		—
Combined (in vitro and in vivo) studies				
Bacteria	Keratitis case	1	8	50
	Laboratory	2		164, 363
	No info.	5		—
Fungi	Laboratory	1	4	2
	No info.	3		—
Protozoa	Keratitis case	1	1	124
Total		125	125	—

Clinical (not ocular), the pathogens have a clinical origin (e.g., an infected wound) but were not harvested from the cornea; Laboratory, the pathogens originate from a pathogen bank or are reference pathogens; No info., the authors of the publication did not indicate the origin of the pathogens.

Records can be found at the supplementary materials in Supplementary Table S1 under the following ID numbers: A: 167, 166, 165, 144, 101, 96, 70, 52, 51, 48, 310, 318, 319, 320, 324, 325, 348, 354, 357; B: 116, 106, 103, 100, 65, 32, 311, 331; C: 208, 206, 123, 110, 71, 59, 191, 333, 353; D: 204, 194, 182, 175, 149, 120, 91.

Rabbits were the most widely used animal species in in vivo laboratory experiments.

Various methods were used to create infectious keratitis animal models in 43 records (full text: n = 34, abstract: n = 9). Intrastromal injection of a pathogen suspension into the cornea ($n = 10$) and corneal epithelium grid/scraping/abrasion, followed by topical application of pathogen solution (n = 10) were the most commonly used techniques (Table 4). Information on the success rate of inducing infectious keratitis in the animal model was provided in six of the 34 full text records. It was successful in 100% of the animals used in four studies[49–52] and in 85.7% and 87.5% in two other studies, respectively.[53,54] The success rate of inducing infectious keratitis in the animal model was not provided in the remaining 28 records. The duration

Table 3. Models Used in PACK-CXL Studies (RQ 3)

Model	N	Record ID
In vitro/ex vivo studies	137	
Suspensions in well/Petrie dish/cuvette	43	A
Porcine corneas	20	B
Suspensions deposited onto plate	20	C
Human corneas	14	D
Human keratocyte culture	7	E
Human corneas—artificial anterior chamber mounted	5	212, 210, 171, 127, 95
Porcine corneas—lamellae*	2	63, 58
Rabbit eye/cornea	2	104, 107
Bovine corneas	2	8, 337
Bovine corneas and biofilm on micro-disc	1	177
Galleria mellonella in glass container†	1	80
Horse/dog/rabbit/porcine eyes	1	1
Plated suspension and epicorneal tissue model	1	167
Porcine corneas—artificial anterior chamber mounted	1	320
Porcine/rabbit/sheep/horse corneas	1	140
Porous polymer with bacteria	1	136
Rabbit/horse corneas	1	155
Rabbit corneas with alkali burn	1	56
Rabbit corneas with closed-loop IOP control system	1	104
Rabbit corneal cell culture	1	182
Suspension in well and biofilm on contact lenses	1	66
Suspension in well and human fibroblast culture	1	14
Suspension in well and plated suspensions	1	52
Suspension in well and porcine corneas	1	19
No info.	6	—
In vivo (laboratory) studies	72	
Rabbit model	57	F
Mouse model	4	169, 13, 3, 365
Rat model	3	118, 4, 322
Rabbit corneas with alkali burn	2	152, 352
Cat model	1	359
Rabbit and chicken model	1	185
Rabbit and rat model	1	192
Rabbit thin cornea model—corneal surface keratectomy	1	21
No info.	2	—
In vivo (animal clinical)		
Client-owned animals	9	G
Combined (in vitro and in vivo) studies	15	
Rabbit model	4	99, 332, 347, 358
Rat model	4	360, 362, 363, 364
Rabbit model and solutions in well/plate	3	164, 50
Client-owned animals	1	304
Hamster model and solutions in well	1	124
Mouse model	1	21
Solutions in well/Petrie dish/cuvette	1	121
Total	233	—

N, sum of records in which the model was used.

Records can be found at the supplementary materials in Supplementary Table S1 under the following ID numbers: A: 204, 201, 199, 197, 175, 149, 144, 123, 120, 116, 110, 106, 103, 102, 101, 100, 91, 85, 79, 78, 67, 65, 64, 51, 48, 46, 33, 32, 30, 28, 23, 200, 191, 181, 180, 310, 311, 319, 353, 354, 367; B: 217, 216, 215, 214, 213, 188, 146, 87, 86, 75, 61, 57, 47, 43, 22, 317, 338, 345, 350, 366; C: 208, 206, 168, 166, 165, 105, 96, 88, 71, 70, 59, 31, 318, 324, 325, 331, 333, 346, 348, 357; D: 218, 211, 209, 189, 125, 109, 94, 76, 73, 53, 93, 327, 355, 356; E: 172, 54, 41, 40, 38, 37, 341; F: 205, 203, 198, 196, 194, 184, 174, 170, 163, 156, 154, 143, 142, 141, 135, 134, 133, 132, 129, 128, 117, 115, 113, 112, 111, 97, 89, 82, 77, 60, 36, 35, 34, 29, 20, 17, 16, 15, 12, 11, 10, 9, 6, 179, 302, 303, 306, 307, 312, 314, 315, 321, 323, 329, 330, 344, 351, 361; G: 161, 160, 138, 137, 74, 45.

*Corneal lamellae with thickness between 150 and 200 µm.

†This record was intentionally classified as in vitro study type although *Galleria mellonella* belongs to an animal kingdom, but considerations regarding reporting, including sex and weight, are not applicable in this case.

Table 4. Methods Used to Create Infectious Keratitis (Bacterial or Fungal) Animal Models (RQ 3)

Method	N	Record ID
Intra/mid-stromal injection of pathogen solution into cornea	10	A
Corneal epithelium grid/scraping/abrasion, followed by topical application of pathogen solution	10	B
Intra/mid-stromal injection of pathogen, followed by corneal epithelium grid/scraping/abrasion	2	306, 307
Creation of 30% depth keratectomy wound, topical application of pathogen solution	1	35
Corneal abrasion, application of infected contact lens, tarsorrhaphy	1	50
Corneal epithelium abrasion, followed by intrastromal injection	1	321
Corneal abrasion, followed by topical application of pathogen solution and tarsorrhaphy	1	322
Suture fixation of acellular cornea, injection of pathogen solution between animal and graft cornea, tarsorrhaphy	1	143
No info.	5	—
Total	34	

Source: records classified as study type '*in vivo* (laboratory) studies' or 'combined (*in vitro* and in *vivo*) studies'.

N: sum of records in which the model was used.

Records can be found at the supplementary materials in Supplementary Table S1 under the following ID numbers: A: 174, 129, 128, 82, 34, 20, 11, 154, 10, 312, 315, 332; B: 169, 164, 118, 13, 2, 365, 347, 360, 362, 363, 364.

between infection and treatment in both fungal and bacterial keratitis animal models ranged from 12 hours to seven days, with the majority of PACK-CXL interventions applied 72 hours after infection (n = 7). Information about the time between infection and treatment was missing in nine full text records.

In vivo studies involving clinical client-owned animal patients with suspected infectious keratitis included dogs, cats, and horses. The following species-appropriate details of animals used in the in vivo studies should be reported according to item 8 (Experimental animals) from the ARRIVE Essential 10 guidelines (the ARRIVE guidelines 2.0: author checklist[55]): species, strain, sex, age, and, if relevant, weight. However, information regarding sex, age, and weight of the animals was missing in 32, 50, and 21 records, respectively, out of 69 full text records in which laboratory animals were used (full text in vivo [n = 56]; full text combined in vivo and in vitro [n = 13]).

Furthermore, item 16 from the ARRIVE Recommended Set guidelines,[55] suggests including a description of any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress in study animals. A description of pain management during the course of the infectious keratitis was provided in two of 34 full text records involving an animal model of induced infectious keratitis, including daily intraperitoneal 4 mg/kg carprofen injections and 0.5% proparacaine hydrochloride eye drops three times daily.[53,56] No information regarding analgesia was provided in 12 of 34 full text records involving an animal model of induced infectious keratitis. According to the information provided in the remaining 20 of 34 records, pain control (systemic analgesia n = 4, topical and systemic analgesia n = 16) was provided only during the "wounding" or PACK-CXL procedures.

RQ4: Which Endpoints Were Used To Assess PACK-CXL-Relevant Treatment Effects?

"Bacterial keratitis" was the largest application domain of the CXL studies that were included into the scoping review (n = 70), with 42 in vitro/ex vivo and 16 in vivo study designs. "Keratoconus/ectatic diseases" (n = 62) and "fungal infections" (n = 39) were the second and third largest application domains, with the majority of records presenting in vitro/ex vivo research results (n = 33 and n =21, respectively) (Fig. 5).

"Antimicrobial effects" and "effectiveness/efficacy against infectious keratitis" were the most broadly researched PACK-CXL-relevant effect categories in the records in which infectious conditions were investigated (included in the application domains "amoebal infections," "bacterial keratitis," and "fungal infections") (Fig. 5A).

One hundred three unique endpoint names relevant to PACK-CXL effects were recorded. Figure 5B represents the heterogeneity of endpoints used in studies

Table 5. Elements Included Into Infectious Keratitis Scoring Systems in 16 Full-Text Records Investigating PACK-CXL Treatment Effectiveness/Efficacy in Infectious Keratitis

		Elements Included in the Keratitis Score by Authors														
					Corneal											
Record Ref.	Name Given to the Score by Authors	Secretion Rating/ Discharge	Conjunctival Hyperemia/ Injection/ Edema	Ciliary Hyperemia/ Uveitis/	Infiltrate	Ulcer/ Epithelial Erosion	Clouding/ Opacity	Edema	Perforation	Melting	Neovascularization	Hypopyon	Blepharitis	Time To Epithelialization	Quoted Original Score Ref.
In vivo (laboratory) studies															
Atalay et al.[57]	Clinical score				•	•		•			•				58
Awad et al.[59]	Clinical signs score		•		•		•				•	•			60
Berra et al.[61]	Clinical score				•		•					•			62
Cosar et al.[63]	Ulcer score				•	•			•			•			None
Elbassiouny et al.[64]	The score of clinical signs		•		•						•	•			None
Galperin et al.[65]	Clinical score/extent of keratitis				•		•					•			62
Kalkanci et al.[66]	Modified Schreiber score		•		•		•				•	•			No info.
Kilic et al.[50]	Clinical examination				•		•								62
Özdemir et al.[67]	Modified Schreiber scale		•		•		•				•	•			60
Peng et al.[68]	Visual scoring system for murine fungal keratitis						•*								None
Pertiwi et al.[69]	Clinical scoring system					•	•	•			•				58,70
Su et al.[53]	Parameters of clinical manifestations		•		•	•					•				None
Tal et al.[71]	Corneal ulcer–related measurements		•		•	•								•	None
Wei et al.[54]	Eye inflammation score	•	•		•		•		•			•			72,73
Wu et al.[74]	Clinical score						•								None
Wei et al.[51]	Schreiber scoring system						•				•				60
Zhang et al.[75]	Observation indexes					•									None
Zhu et al.[76]	Inflammatory index			•			•								77
In vivo (animal clinical) studies															
Famose et al.[28,78]	Modified Tajima clinical score	•	•	•	•	•		•		•	•		•		79
Marchegiani et al.[80]	Modified clinical score	•	•	•				•			•		•		28,78
Spiess et al.[27]				•	•	•				•				•	None
Combined (in vitro and in vivo) studies															
Bai et al.[81]	Slit-lamp score														None
El-Laithy et al.[82]	Signs of infection and inflammation	•	•			•									None
Kashiwabuchi et al.[83]	Clinical scoring criteria					•	•	•			•				84
Shih et al.[85]	Clinical score						•*								58
Wang et al.[86]	Clinical score														87
Zhang et al.[88]	Clinical score	•	•		•							•			None
Zhou et al.[89]	Corneal clinical score						•*								None
Zhu et al.[90]	Schreiber scoring system					•	•				•				62
Total	29	5	11	3	15	11	16	5	2	2	12	9	2	2	

*Area of corneal opacity, density of opacity, and surface regularity

Infiltrate: presence, diameter, size; thickness; Corneal ulcer/epithelial erosion: diameter, size, area, depth; Hypopyon: altitude, level, mm.

focused on determining the antimicrobial effects of PACK-CXL in the application domains "amoebal infections," "bacterial keratitis," and "fungal infections." "Acanthamoeba count," "bacterial elimination [CFU or CFU/m"]" and "fungal growth inhibition" were the most frequently used endpoints in these application domains (Fig. 5B).

Infectious keratitis scoring was the most frequently used endpoint to assess PACK-CXL treatment "effectiveness/efficacy in infectious keratitis" in amoebal, bacterial, and fungal infection studies (Supplementary Fig. S3). Table 5 presents the existing heterogeneity in infectious keratitis scoring systems across 29 full text records (in vivo [laboratory] studies n = 18; in vivo [animal clinical] studies n = 3; Combined [in vitro and in vivo] studies n = 8), especially regarding the explanation of definitions and elements used in the scoring systems. "Corneal opacity/clouding" was used the most consistently across all scoring systems and was part of the scoring system in 16 out of 29 records. Detailed information was often missing from the records, precluding the use of the same scoring systems in future studies with similar applications. For example, some record methods stated that ulcer size was measured, but failed to define the criteria used for ulcer size grading which was how the data was presented in the results section. In other records, these measurements were not presented at all in the results section. Additionally, an explanation of the choice of elements included in the infectious keratitis scoring system that was used in the study, and its relevance to clinical cases, was typically missing from the records.

A large heterogeneity was observed regarding the definition of endpoints used to measure the antimicrobial effects of PACK-CXL (Fig. 5B), including the methods of quantification, measurement timepoints and SI units used in the original records. The same level of heterogeneity was observed for the other investigated PACK-CXL-relevant effect endpoint categories (resistance to enzymatic tissue degradation, treatment penetration depth, cellular/morphological response, effectiveness/efficacy in infectious keratitis, other effects), which were mapped in Supplementary Figure S3.

Discussion

This scoping review demonstrated that preclinical research into the antimicrobial and tissue stabilizing effects of PACK-CXL is a large and diverse field. Many PACK-CXL protocol modifications are being explored towards the elimination of clinically relevant infectious agents in both in vitro/ex vivo and in vivo studies. Two major problems were observed that preclude the conduction of a meta-analysis, evaluation of the strength of evidence, and the subsequent translation of existing research results to clinical practice. The first major problem is widespread shortcomings in the reporting of research designs and results, which can only partially be explained by the inclusion of both conference abstracts and full text manuscripts in the review. The second major problem is the large heterogeneity of experimental methods and the lack of consensus on the common most relevant endpoints for infectious keratitis. Those shortcomings slow down advancement in the field of PACK-CXL research and lead to an ineffective use of resources.

A scoping review is a form of knowledge synthesis that addresses an exploratory research question aimed at mapping key concepts, types of evidence, and research gaps related to a defined area or field by systematically searching, selecting, and synthesizing existing knowledge.[91–93] A scoping review does not analyze data to answer a narrow research question. Instead, it provides a broad overview of what has been published in a field.[91] Arksey and O'Malley[92] proposed the first framework for scoping reviews, which was further developed by various authors.[40,91,94,95] A summary of the available methodology was recently published through the Joanna Briggs Institute.[96] In addition, the PRISMA Extension for Scoping Reviews (PRISMA-ScR) provides an item list to improve the reporting quality of scoping reviews.[41]

Despite its rigor, this review also has several notable limitations. For example, the inclusion of records without a primary focus on infectious keratitis largely depended on the reviewers' judgment of the relevance of these studies to infectious keratitis treatment. The authors attempted to reduce personal bias through the use of three questions to determine record eligibility for inclusion (see Methods section, Selection of studies). The exclusion of endpoints only relevant to the biomechanical effects of CXL treatment, and thus deemed to be relevant mainly for the treatment of keratoconus and other ectatic disorders, and not infectious keratitis, also depended on the reviewers' judgment. The grouping of PACK-CXL studies into application domains and effect categories, and the grouping of original endpoint descriptions under common endpoint names, may also have been influenced by personal decisions. The authors therefore acknowledge that other choices regarding grouping would have been possible. Additionally, one person was responsible for data extraction. Finally, the results were presented at an overview level, and many interesting subanalyses were not conducted. The possibilities for data analy-

sis on this and similar datasets are therefore far from exhausted, and the authors hope that this work will inspire and enable a deeper investigation into the topics discussed here.

The authors acknowledge that the reporting of study design and results is a complex undertaking and that some items can easily be overlooked. However, when reporting guidelines are readily available, errors may be avoided. Based on the review, the authors have identified four areas relevant to study reporting that could be improved to enable knowledge synthesis and to secure the reproducibility of scientific studies or results. These areas are presented below.

1) None of the included records fully adhere to the selected items from the ARRIVE guidelines,[44,55] which provide an easy-to-use reporting system with checklists for animal experimentation reporting. These guidelines are available online and should be considered as a minimum standard for study reporting. In the context of reproducibility of infectious keratitis animal models, the authors further suggest the inclusion of the following information: the pathogen load (total amount, concentration) used to induce disease, the method of wounding, and the time between induction of infection and treatment start.
2) Treatment and maintenance of experimental animals in accordance with the ARVO Statement was claimed in most records.[43] However, especially in the context of infectious keratitis, a painful disease, it is unsettling that the information provided on pain control strategies was incomplete in many records. Information on pain control was not supplied in 12 of 34 full text records involving an animal model of induced infectious keratitis. According to the information provided in 20 of 34 full text records, pain control was provided only during the "wounding" or PACK-CXL procedure. Four records described a three-week follow-up period during which some animals developed corneal perforations. Additionally, in two full-text records in the "keratoconus/ectatic disorders" domain, topical antibiotics were listed as pain control medications. The authors assume that the fact that pain control information was missing from many records means that the information was not provided and not that pain control itself was not provided during the observation periods after the induction of corneal infections and treatment with PACK-CXL. However, authors and reviewers should be aware of existing reporting standards regarding pain control strategies and pain scoring systems in experimental animals, which can prevent omissions of the information supplied in published records.
3) Details regarding the intervention (PACK-CXL protocol) were insufficiently reported. The biggest gaps were observed in the reporting of chromophore saturation time, total dose of delivered energy (fluence), and irradiation duration or intensity. Chromophore concentrations were reported using various SI units, which complicates overall treatment effect quantification across different protocols used. Information detailing PACK-CXL protocols used is crucial for the knowledge synthesis that is necessary to direct translational research.
4) Despite the adoption of the term PACK-CXL in 2013 by clinical opinion leaders in the field, many synonyms and alternative terms continue to be used in publication titles and abstracts, which may hinder knowledge synthesis by preventing relevant publications to easily be identified. Since PACK-CXL is an abbreviation and the full name is long, it may be considered an unpractical name for usage in titles.

Apart from incomplete study reporting, the large heterogeneity of reported endpoints is another problem precluding quantitative knowledge synthesis. It was difficult to extract the outcomes of interest and their corresponding endpoint measurements from some of these published records. Ting et al.[30] observed that clinical trials and case series on PACK-CXL treatment of human patients with infectious keratitis suffered from a similar heterogeneity in outcome reporting. The Core Outcome Measures in Effectiveness Trials (COMET) initiative[97] defines a core outcome set as an agreed standardized set of outcomes that should be measured and reported, as a minimum, in specific health or healthcare domains. The authors believe that the establishment of clinically relevant core outcome sets for infectious keratitis, which would reflect different PACK-CXL treatment effects, would address the large reported endpoint heterogeneity in PACK-CXL-related research and thus benefit the field of PACK-CXL.

An interdisciplinary approach involving clinicians, epidemiologists, and basic scientists would be needed to define core outcome sets for preclinical and clinical PACK-CXL studies. With such core outcome sets in place, authors that aim to publish in the field of PACK-CXL would be expected to have collected and reported the relevant core outcome sets, without having to restrict their outcome measurements solely

to the core set. This would facilitate the comparison of results while allowing researchers to continue exploring additional outcomes as well.

The semiquantitative preclinical ocular toxicology scoring (SPOTS) system is an established scoring system that is available to be adapted and used for preclinical in vivo infectious keratitis studies.[98] This system provides scoring criteria for the anterior and posterior segment, and focuses on the standardization of examination procedures and scoring criteria for corneal and anterior segment pathology. Adaptation and adoption of the SPOTS system could limit discrepancies between currently used keratitis scoring systems, which were illustrated in Table 5.

Assessing risk of bias was not part of this scoping review because this is typically conducted in systematic reviews. However, the authors point out that nonsignificant results were reported in only three of the 233 records included. It is therefore likely that a bias toward the publication of significant results is present in the preclinical PACK-CXL research field.[99,100]

In conclusion, a quantitative knowledge synthesis of the antimicrobial and tissue stabilizing effects of PACK-CXL, as in a meta-analysis or systematic review, is impossible despite the wide range of PACK-CXL protocol modifications that have been explored in in vitro/ex vivo and in vivo preclinical studies. Incomplete reporting and the large heterogeneity of reported endpoints are the two major problems that slow down advancement in the field. Harmonization through the establishment of core outcome sets is urgently needed.

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* SAP and SH contributed equally to the manuscript and therefore share last authorship.

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