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Drug nanocrystals: A review of their application in the topical treatment of skin infections

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Review Paper

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Abstract:

Drug nanocrystals, nano-sized delivery systems consisting of drug and stabilizers only, may be a valuable tool in the treatment of cutaneous infections, which remain a serious public health problem worldwide. This review aimed to compile scientific information published over the past 10 years on the topical use of nanocrystals for the treatment of cutaneous infections caused by fungi or bacteria (14 selected articles). Most articles synthesized nanocrystals by a top-down method, mainly the bead milling technique. After synthesis, nanocrystals were typically lyophilized and/or incorporated into a pharmaceutical formulation. The most used techniques for nanocrystal characterization were dynamic light scattering (particle size and polydispersity), electron microscopy (ultrastructural morphology) and high-performance liquid chromatography (solubility, dissolution, and drug release). A temperature of 4 °C was deemed optimal for storage. Several articles found that nanocrystals improved drug skin permeation and/or skin retention. Four articles conducted tests with fungi, nine articles with bacteria, and one article with fungi and bacteria, either in vitro or in vivo. In general, microbiological tests confirmed the greater effectiveness of nanocrystal formulations over conventional systems. The findings of this review demonstrate the potential of nanocrystals as safe and promising alternatives for the topical treatment of cutaneous infections caused by fungi or bacteria.

Keywords: Bacteria; Cutaneous; Fungi; Infection; Nanocrystals; Nanotechnology; Skin

1. Introduction

Skin infections represent a serious public health problem worldwide. Invasion of the skin by microorganisms (fungi, bacteria, or viruses) induces a local inflammatory response manifested by pain, erythema, edema, and sensitivity to palpation and heat. Systemic symptoms such as fever, chills, and malaise can also be observed. The severity of skin infections depends on how deeply microorganisms reach into skin layers. The resulting lesions can be papular, nodular, ulcerative, or blistering, and may develop a purulent appearance over time [1].

In general, topical therapy is the preferred initial approach for treating cutaneous infections. However, it is challenging to effectively deliver active principles to the various layers of the skin [2]. Nevertheless, topical administration is associated with several advantageous characteristics, such as high patient compliance, low incidence of systemic side effects, reduced drug interactions, absence of first-pass metabolism, and high drug concentrations at the site of application [3, 4].

Nanotechnology serves as a valuable tool in developing potent and effective topical treatments of skin infections. Several types of nanoparticles have been successfully used for this purpose, including polymeric nanoparticles [5–7], lipid nanoparticles [8, 9], vesicular nanoparticles [10, 11], metal nanoparticles [12, 13] and, more recently, nanocrystals [14].

Nanocrystals are particles with a size between 100 and 1000 nm. They consist of drug and stabilizing agents, such as surfactants and/or polymers, which are distributed along the crystal surface. Nanocrystals are known for their high surface area, which increases the saturation solubility and the

drug dissolution, therefore increasing bioavailability of hydrophobic drugs via several administration routes [15, 16]. Nanocrystals technology can be applied in cutaneous drug delivery, as it may increase drug loading capacity, promote passive diffusion, enhance skin adhesion and facilitate penetration into the skin tissue [16]. The physicochemical properties altered by the nano-crystallization process - such as increased drug kinetic solubility and surface area - play a key role in promoting enhanced interaction with the stratum corneum [16, 17]. Additionally, nanocrystals require minimal amounts of excipients and vehicles and present low toxicity, ease of preparation and scale-up, and low production cost [2–4, 18].

Especially considering skin infections, a high dose of antibacterial agent is needed at the site of infection, which is difficult to achieve with conventional topical medications. Those treatments are frequently long-lasting and/or with high dosages [17]. In this context, topically applied nanocrystals can enhance the delivery of the drugs to the infected tissue and prolong drug release [14], potentially decreasing the frequency of applications, the needed dosage, the chances of recurrence and the side effects.

In view of the foregoing, this study compiled scientific information published over the past 10 years on the use of nanocrystals for the treatment of skin infections caused by fungi or bacteria, with the aim of synthesizing and discussing the main results of these studies. To the best of our knowledge, despite the reviews that are available on the application of nanocrystals in dermatologic diseases [14, 16, 17, 19], this is the first review article focusing exclusively on skin infections and drug nanocrystals.

2. Composition and techniques for nanocrystal synthesis and formulation

Table 1 presents the materials and methods used in the selected studies to synthesize nanocrystals and nanocrystal formulations. Of the 14 selected articles, nine developed nanocrystals containing antibacterial compounds, four developed nanocrystals containing antifungal compounds, and one developed nanocrystal containing antibacterial and antifungal drug (chloroxine) [30]. Only one study used a natural substance as antimicrobial (kaempferol, a flavonoid) [28], whereas the other 13 articles focused on drugs with a long history of use in the treatment of skin infections. Drug concentrations in nanocrystals ranged from 0.003% to 10% [4, 20–27, 29–32].

One of the key principles for obtaining nanocrystals is that the active substance must be poorly soluble in the medium [18]. Nanocrystals contain only the drug and stabilizing agents, such as surfactants and/or polymers. Stabilizers enhance the physical stability of the formulation by forming an interfacial barrier that prevents particle aggregation and crystal clustering during storage [2, 33]. Several studies explored the application of different stabilizers and chose the most suitable for in-depth analysis. Non-ionic surfactants, such as polysorbate 80, are preferable for preparing topical formulations of nanocrystals [2, 26, 32].

The methods used for nanocrystal synthesis in the selected articles are illustrated in figure 1. The top-down methods involve the reduction of particle size from coarse suspensions to nanometric particles, using high energy input. The main techniques used for this purpose are media milling and high-pressure homogenization [34], which are the preferred methods of nanocrystal production in the pharmaceutical industry [34, 35]. Advantages of media milling include the reduction of size in a reproductible manner, while disadvantages include long operation time and possibility of contamination from the beads. Regarding high-pressure homogenization, advantages include ease of scale up, while disadvantages include the need of a complex equipment [16]. Of the selected articles, five used media milling techniques for nanocrystal synthesis [4, 20, 21, 27, 29]. Of note, Permana and co-workers (2020) and Anjani and co-workers (2022) used the small-scale method developed by Romero and co-workers (2018), with some modifications [27, 29, 36]. Two articles used high-pressure homogenization [24, 25]. Azelaic acid nanocrystals were developed by media milling and high-pressure homogenization, and average sizes were 200 nm and 500 nm, respectively [32].

On the other side, bottom-up methods are based on controlled nanoprecipitation from a drug solution, and present advantages such as simple instrumentalization, low energy and low heat generation [34]. The major drawback of this approach is the frequent use (and need for removal) of organic solvents [34]. Of the selected articles, six used a bottom-up method (nanoprecipitation) for nanocrystal synthesis [22, 23, 26, 28, 30, 31].

Nanocrystals are usually obtained as a liquid aqueous suspension. The suspension is then subjected to drying to increase the stability of nanocrystals and reduce problems associated with liquid samples [37]. Of the selected articles, almost half (6 articles) used freeze-drying as post-synthesis treatment [22, 26, 28, 29, 31, 32]. In one study, nanocrystals were left to dry at room temperature [23].

Different vehicles can be used for the topical release of nanocrystals. For nanocrystals to be effectively incorporated into a pharmaceutical formulation, they must be present at a concentration greater than their solubility; otherwise, they would be solubilized by the medium. Additionally, no, or minimal aggregation should be observed [2]. Of the selected articles, six incorporated nanocrystals into gels [4, 20, 21, 23–25], two into creams [26, 31], two into microneedles [27, 29], and one into films [28]. By a previous study on hesperidin nanocrystals, it was observed that cream and oleogel were more suitable for the incorporation of lipophilic substances in the form of nanocrystals, considering the influence of the vehicle on the skin penetration of the drug [38].

3. Physicochemical properties of nanocrystals

Table 2 shows the physicochemical characteristics of nanocrystals and the main results of the cited research. All studies conducted particle size determination assays, and dynamic light scattering was applied in 13 articles, and one article used the laser diffraction technique to evaluate particle size [26]. A wide range of diameters was reported (from < 100 to 580 nm); however, most nanocrystals fell within the $200 - 300$ nm range. The polydispersity index (PDI),

Table 1. Drugs, materials, and methods used for the synthesis of nanocrystals for the treatment of skin infections.

Drug and concentration	Stabilizer	Method	Post-synthesis treatment/pharmaceutical formulation	Reference
0.1% silver sulfadiazine	Chitosan	Wet bead milling	Chitosan hydrogel	[20]
1% miconazole	Polysorbate 80 and poloxamer 407	Wet bead milling	Hydroxypropyl cellulose gel	[21]
1% fusidic acid	Poloxamer 188	Nanoprecipitation followed by sonication	Lyophilization	[22]
0.5% luliconazole	Vitamin E TPGS and HPMC	Nanoprecipitation followed by sonication	Drying at room temperature/carbopol hydrogel	[23]
10% silver sulfadiazine	Poloxamer 407	High-pressure homogenization	Thermoresponsive hydrogel	[24], [25]
2% clotrimazole	Polysorbate 80	Wet bead milling	Carbopol hydrogel	[4]
2% fusidic acid	PVA 4-88	Nanoprecipitation followed by high-speed homogenization	Lyophilization/cream	[26]
2% itraconazole	Pluronic® F127	Wet bead milling	Microneedles	[27]
Kaempferol (dosage not informed)	Sodium dodecyl sulphate	Nanoprecipitation followed by sonication	Lyophilization/polyhydroxybutyrate chitosan film	[28]
0.003% metronidazole	Soluplus®	Wet bead milling	Lyophilization/microneedles	[29]
0.01% chloroxine	Brij 700	Nanoprecipitation	Not reported	[30]
1% mupirocin	PVA and K-P188	Nanoprecipitation followed by high-speed homogenization	Lyophilization/cream	[31]
5% azelaic acid	Polysorbate 80	Wet bead milling and high-pressure homogenization	Lyophilization	[32]

which indicates formulation homogeneity, ranged from 0.09 to 0.39. It is important to note that no studies reported PDI values greater than 0.4, possibly indicating narrow size distributions [21]. In addition to dynamic light scattering, other techniques can be used to characterize nanocrystals (figure 2).

The zeta potential represents the electrostatic charge of the particle surface. Particles can be classified as highly unstable ($\pm 0 - 10$ mV), relatively stable ($\pm 10 - 20$ mV), moderately stable ($\pm 20 - 30$ mV), and highly stable (± 30 or greater) according to their zeta potential [2]. Among the selected articles, seven reported zeta potential values ($|11|$ to $|39|$ mV), as measured by electrophoretic mobility [21–23, 26, 28, 30, 31]. Zeta potential determination was performed in purified water in most cases and in conductivity-adjusted water (50 µS/cm and pH 5.5) [21]. It is important to mention that not only electrostatic, but also the steric effect can play an important role in the stabilization of nanocrystals [21].

Analytical methods (UV or HPLC-UV) were applied for the measurement of drug content, solubility, and drug release. Several articles reported an increase in the saturation solubility of nanocrystal samples, usually compared with that of a coarse powder [4, 22, 23, 26, 31] or coarse suspension [4, 26, 31]. Eight articles evaluated the drug release profile of nanocrystals [4, 20, 24, 26–29, 31]. In these studies, the drug release rate of semisolid nanocrystal formulations was higher than that of commercial formulations, probably

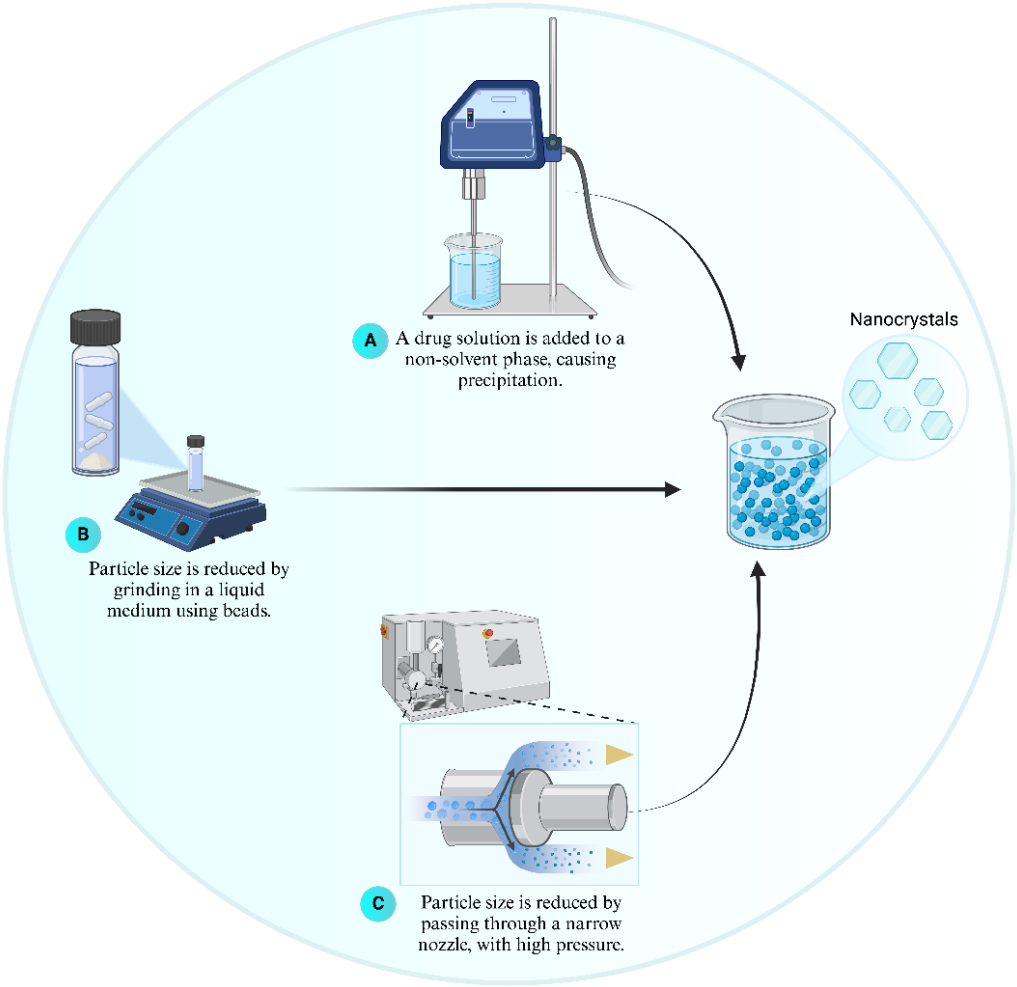

Figure 1. Methods commonly used for nanocrystal synthesis. (A) Nanoprecipitation; (B) Small-scale bead milling; (C) High-pressure homogenization.

because of their enhanced solubility and faster dissolution [4, 20, 24, 26, 31].

Electron microscopy allows evaluating the morphology and characteristics of nanoparticles, in addition to predicting the behavior, stability, and efficiency of particles [39]. Thirteen articles used electron microscopy (either scanning or transmission electron microscopy) to investigate nanocrystal morphology [4, 20, 22–32]. Pyo and co-workers (2017) used light microscopy to investigate the presence of microcrystals and potential aggregates in nanocrystal formulations [21].

Other techniques to evaluate nanocrystals include differential scanning calorimetry (DSC) and X-ray diffraction (XRD), used to determine melting and crystallization behavior [2, 4, 22, 23, 26, 29, 31, 32]. Fourier transform infrared spectroscopy (FTIR) can be used to determine interactions between nanocrystal components and chemical changes during formulation [4, 22, 23].

Eight articles studied the storage stability of nanocrystals [4, 20–24, 26, 31]. Several authors concluded that the best storage temperature was 4 °C [21, 22, 26, 31]. The adsorbed stabilizer may not function properly when subjected to temperature stress [31]. Freeze-drying nanocrystals may enhance stability in terms of particle size and size distribution [22, 26]. Gao and co-workers (2016) observed that incorporation of genipin nanocrystals into chitosan hydrogels prevented an increase in particle size [20].

4. Skin permeation/penetration of drugs from nanocrystals

Drug molecules can follow three routes to penetrate the skin: the intracellular or transcellular route (through keratinocytes of the stratum corneum), the intercellular pathway (across spaces between cells), and the follicular pathway [40]. Figure 3 depicts the routes of skin penetration of drugs when applied in the form of nanocrystals. Penetration can occur through the skin (via intra- or transcellular routes), due to high passive diffusion resulting from increased saturation solubility and concentration gradient, and through hair follicles, forming a drug reservoir that can easily reach even deeper skin layers [2, 15]. It is important to consider that such scenario considers the intact skin and nanocrystals larger than 100 nm in diameter. However, when the skin has its barrier impaired or when the nanocrystals present diameters smaller than 100 nm, the nanoparticles may be able to reach viable skin layers, which can lead to an even higher increase in the drug skin penetration/permeation [17, 41].

Nine of the selected articles performed *ex vivo* penetration or permeation studies. In most of these studies, nanocrystals were previously incorporated into the final pharmaceutical

Table 2. Physicochemical properties of nanocrystals for the treatment of skin infections.

Drug and concentration	Average diameter (nm)	Polydispersity index (PDI)	Zeta potential (mV)	Stability	Reference
0.1% silver sulfadiazine	290	0.240 to 0.250	Not reported	42 days at room temperature (hydrogel)	[20]
1% miconazole	346 (polysorbate 80); 355 (poloxamer 407)	0.39 (polysorbate 80); 0.36 (poloxamer 407)	39 (polysorbate 80); 21 (poloxamer 407)	90 days at 4 °C (higher stability for poloxamer 407 nanocrystals, chlorhexidine digluconate minimized crystal growth)	[21]
1% fusidic acid	265	0.158	-16.9	90 days at 4 °C and room temperature (wet and lyophilized formulations)	[22]
0.5% luliconazole	263	0.093	-18.36	7 days at 2 °C	[23]
10% silver sulfadiazine	About 250	About 0.250	Not reported	6 months at 4 °C	[24]
10% silver sulfadiazine	292	0.288	Not reported	6 months at 4 °C (according to Liu et al.,[24])	[25]
2% clotrimazole	264	0.211	Not reported	Not reported	[4]
2% fusidic acid	138	1.3 (span value)	-11.6	30 days at room temperature and 4 °C	[26]
2% itraconazole	352	0.37	Not reported	Not reported	[27]
Kaempferol (dosage not reported)	145	0.243	-31	Not reported	[28]
0.003% metronidazole	46.7	0.09	Not reported	Not reported	[29]
0.01% chloroxine	580	Not reported	-18.9	Not reported	[30]
1% mupirocin	69.7 (PVA); 74.9 (K-P188)	0.22	-15.1 (PVA); -12.6 (K-P188)	90 days at room temperature and 4 °C	citeu30
5% Azelaic Acid	246 and 500	0.32 and 0.25	Not reported	Particle size remained unchanged after 24 h of preparation	[32]

form. The results indicated that the use of nanocrystals increased drug retention in the skin, as compared with non-nanometric control formulations [4, 21, 23, 26]. The studies also reported lower permeation to the receptor compartment [4, 23], which can lead to lower systemic drug absorption. This might have occurred because of the increased saturation solubility, dissolution rate, and skin adhesion (increasing contact time) provided by nanocrystals. Furthermore, nanocrystals can be distributed across hair follicles [23, 26]. An increase in skin retention and penetration may improve drug effectiveness, as it reduces the number of daily applications, improves the local effect, and decreases systemic action and cytotoxicity [2, 26, 31].

Najm and co-workers (2022) suggested that drug skin permeation and retention are dependent on nanocrystal particle size. Smaller particles can lead to enhanced skin permeation and reduced skin retention [31]. Pyo and co-workers (2018) confirmed the effect of the surfactant in the drug skin penetration from nanocrystals [21]. Permana and co-workers (2020) observed that the use of microneedles as the final pharmaceutical form for itraconazole nanocrystals improved drug penetration into the epidermis and dermis compared with a needle-free patch containing nanocrystals and a nanocrystal cream [27]. It has been previously demonstrated that the excipients, the skin hydration as well as the vehicle selected for the incorporation of nanocrystals, can greatly influence skin penetration [38].

Ji and co-workers (2024) evaluated the penetration of azelaic acid in the skin and hair follicles [32]. The nanocrystals (200 and 500 nm) exhibited a higher ability for penetration into the hair follicles, compared to the coarse suspension and the commercial product, in a way that the 500 nm nanocrystals exhibited the highest follicular penetration capacity [32]. The authors suggested that this enhancement

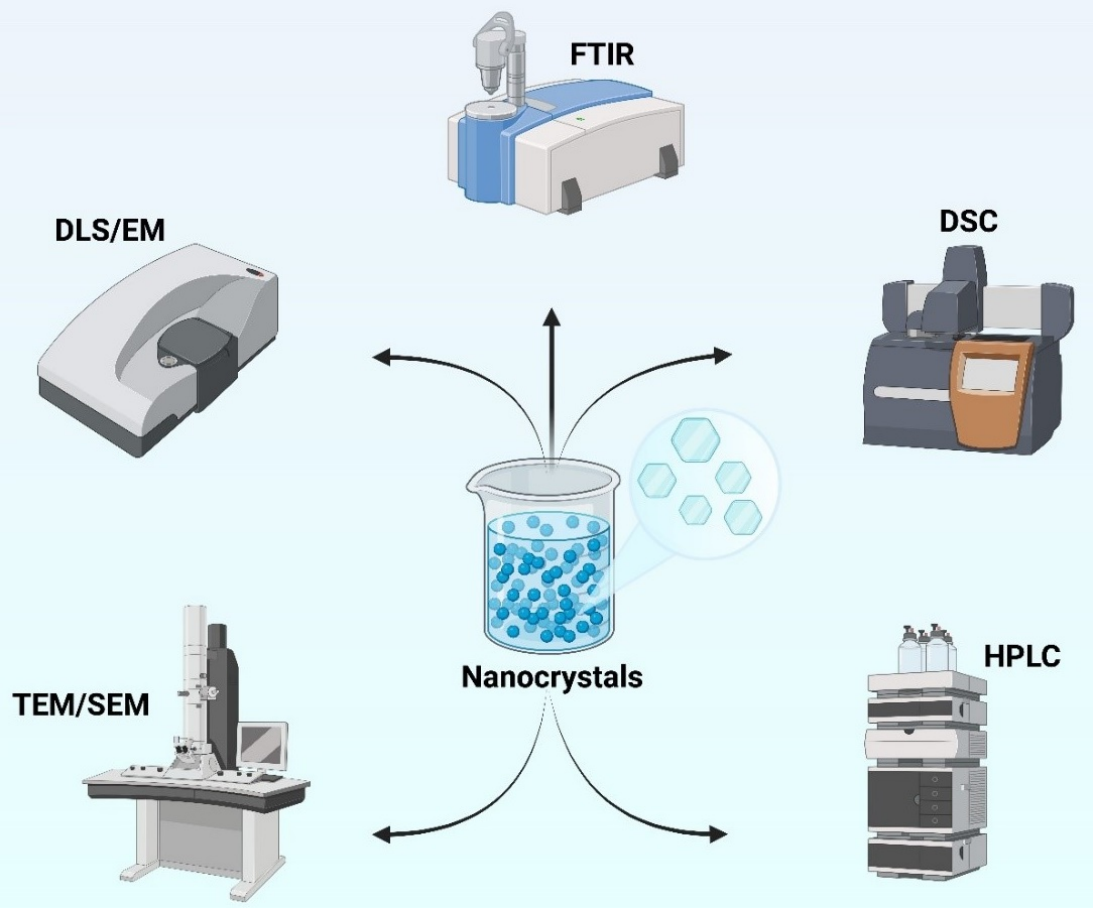

Figure 2. Techniques commonly used for nanocrystal characterization. DLS = dynamic light scattering; EM = electrophoretic mobility; FTIR = Fourier-transform infrared spectroscopy; DSC = differential scanning calorimetry; TEM = transmission electron microscopy; SEM = scanning electron microscopy; HPLC = high-performance liquid chromatography.

may be attributed to particle size, as particles in the range of 400 – 700 nm tend to accumulate within the follicles [32]. Regarding the stratum corneum, no differences were observed, and the azelaic acid was not quantified in the other skin layers. The influence of radiofrequency and ultrasound in combination with nanocrystals on the hair follicular penetration of azelaic acid was also evaluated. When using ultrasound, the authors observed a considerable increase in

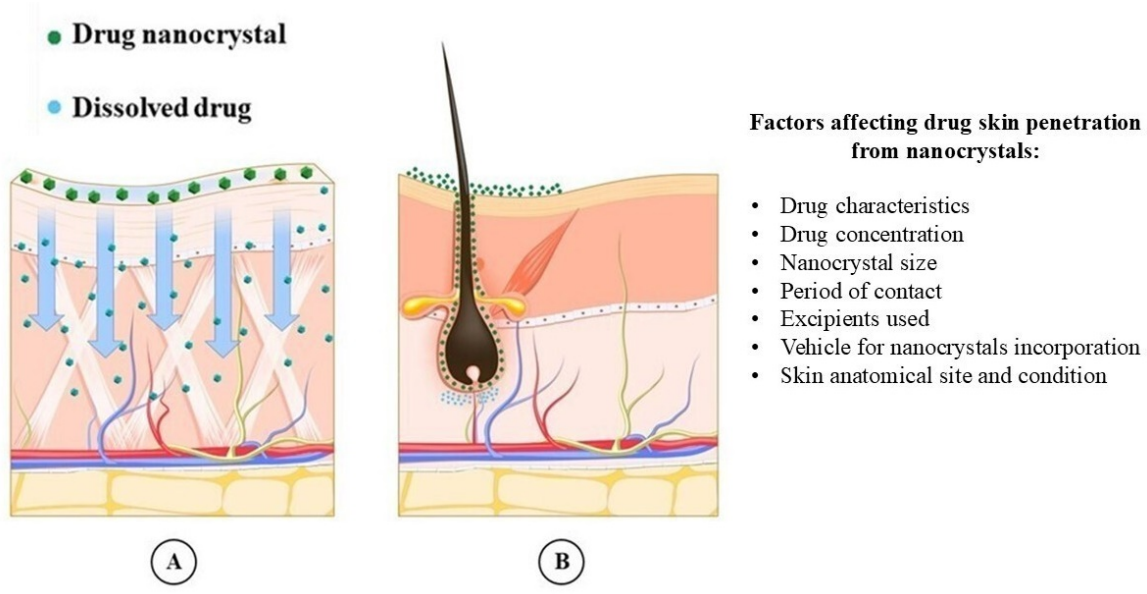

Figure 3. Penetration routes of drugs from nanocrystals and factors affecting drug penetration. (A) Intra- or transcellular routes; (B) Follicular penetration.

the ability of azelaic acid to penetrate the hair follicles [32].

5. *In vitro/in vivo* studies with bacteria and fungi

The *in vitro* and *in vivo* studies are described in Table 3. *In vitro* tests were conducted in all selected articles. The assays comprised the inhibition zone test and minimum inhibitory concentration (MIC) determination. Six articles used rat or mouse infection models [4, 20, 22, 24, 26, 31], one used a rabbit acne model [32], and one article utilized an *ex vivo* infection model on porcine skin [27].

Among the selected articles, five conducted tests with fungi, including *Candida albicans* [4, 21, 23, 27, 30] and *Malassezia* spp. [30]. The authors observed that nanocrystal formulations promoted an increase in the inhibition zone compared with drug dispersions [4, 21, 23] and commercial formulations [4, 21]. Furthermore, the tested formulations had a lower MIC on fungi than the control drug dispersion [27]. The results of time–kill assays demonstrated that the killing rate of *C. albicans* by itraconazole nanocrystals is

Table 3. Microbiological assays performed for nanocrystals for skin infections.

Drug and concentration	Microorganisms	*In vitro* test *Ex vivo* and *in vivo* tests	Reference	
0.1% silver sulfadiazine	*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*	Inhibition zone	*In vivo* excision and cutaneous burn wound mouse model	[20]
1% miconazole	*Candida albicans*	Inhibition zone	Not reported	[21]
1% fusidic acid	*Staphylococcus aureus* and methicillin-resistant *S. aureus*	MIC	*In vivo* skin-infected mice	[22]
0.5% luliconazole	*C. albicans*	Inhibition zone	Not reported	[23]
10% silver sulfadiazine	*S. aureus*, *E. coli*, and *P. aeruginosa*	MIC, MBC, and inhibition zone	Not reported	[24]
10% silver sulfadiazine	*S. aureus*, *E. coli*, and *P. aeruginosa*	MIC, MBC, and inhibition zone	*In vivo* wound healing studies in mice	[25]
2% clotrimazole	*C. albicans*	Inhibition zone	*In vivo* antifungal activity in rats	[4]
2% fusidic acid	*S. aureus* and *Staphylococcus epidermidis*	Inhibition zone, MIC, and MBC	*In vivo* rat excision wound infection model	[26]
2% itraconazole	*C. albicans*	MIC, MFC, and time-kill assay	*Ex vivo* fungal infection model on porcine skin	[27]
Kaempferol (dosage not reported)	*S. aureus*, *Enterococcus faecalis*, *P. aeruginosa*, *Acinetobacter baumannii*, *Bacillus subtilis*, *Klebsiella aeruginosa*, and *E. coli*	MIC, inhibition zone, and bacterial viability	Not reported	[28]
0.003% metronidazole	*Bacteroides fragilis*	Inhibition zone	Not reported	[29]
0.01% chloroxine	*A. baumannii*, *Bordetella bronchiseptica*, *Corynebacterium pseudodiphtheriticum*, *Cronobacter sakazakii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *P. aeruginosa*, *Shigella flexneri*, *S. aureus*, *S. epidermidis*, *Serratia marcescens*, *C. albicans*, *Malassezia furfur*, and *Malassezia pachydermatis*	MIC, MBC and MFC	Not reported	[30]
1% mupirocin	*S. aureus*	Inhibition zone, MIC, and MBC	*In vivo* wound healing assay in a rat burn wound model	[31]

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration.

concentration dependent [27].
Using an *ex vivo* test (fungal infection model on porcine skin), Permana and co-workers (2020) concluded that the combination of two delivery systems (microneedles and nanocrystals) could enhance the skin penetrability of the drug, decreasing the killing time and reducing fungal burden compared with the needle-free itraconazole patch and the commercial cream [27]. Patel and co-workers (2019) induced *C. albicans* infection in albino Wister rat skin and observed that, after 12 days of treatment, clotrimazole nanocrystals afforded a notable reduction in the cutaneous bioburden of fungal infection; these results were achieved faster than with a commercial formulation [4]. This was the only article that evaluated antifungal activity *in vivo*.
Ten of the 14 selected articles carried out tests with bacteria. Almost all studies used *Staphylococcus aureus* [20, 22, 24–26, 28, 30, 31]. Nanocrystal formulations increased the inhibition zone compared with fusidic acid [26] and mupirocin [31] commercial formulations and compared with the bulk form of silver sulfadiazine [25]. Some authors observed a decrease in MIC values with the use of nanocrystal formulations in comparison with coarse powder [22, 24, 25, 28]. On the other side, azelaic acid nanocrystals were evaluated regarding their *in vitro* antibacterial effect against *Cutibacterium acnes*, in comparison with different antibacterial solutions [32]. The MIC of the azelaic acid nanocrystal was the lowest among all the experimental groups tested (Pionin, Punica Granatum, Rhodiola Rosea), which was probably related to the reduction in the intracellular pH caused by the active substance [32].
Six of the ten studies with bacteria conducted *in vivo* tests [20, 22, 25, 26, 31, 32], being two with silver sulfadiazine and two with fusidic acid. Gao and co-workers (2016) observed that a hydrogel formulation containing silver sulfadiazine nanocrystals promoted greater wound contraction (burn wound) than hydrogels containing bulk powder and silver sulfadiazine commercial cream [20]. More recently, Liu and co-workers (2023) infected mice with *S. aureus* to evaluate the wound healing rate of silver sulfadiazine nanocrystals with thermosensitive hydrogel [25]. The study demonstrated that the developed formulation resulted in faster healing than the commercial cream, with a significant reduction in bacterial load [25]. A patent has been described with the drug silver sulfadiazine in the form of nanocrystals, which was nano-crystallized by ball milling. The nanocrystals presented, at a concentration of 0.5%, the same efficacy of a 1% silver sulfadiazine conventional cream [17].
Omolo and co-workers (2018) administered an intradermal injection of *S. aureus* Rosenbach (ATCC BAA-1683) (MRSA) to cause infection within the dermal layer of the animal's skin, without systemic infection. The authors found that the fusidic acid nanocrystal formulation was more efficient than the fusidic acid DMSO solution [22]. Ahmed and co-workers (2020) infected an animal wound with topical application of 15 µL of *S. aureus* (ATCC29737) to the dermis, without causing systemic infection. The authors reported that the cream containing lyophilized fusidic acid nanocrystals improved the distribution of the active ingredient in the infected wound, resulting in more effective therapy in comparison with the commercial formulation [26].
Najm and co-workers (2022) infected a burn wound with S. aureus, and the mupirocin nanocrystal cream exhibited greater efficacy than the commercial ointment, showing rapid and complete healing [31]. Ji and co-workers (2024) evaluated the azelaic acid nanocrystals efficacy in a composite acne model induced by intradermal injection of *Cutibacterium acnes* and topical application of 30% oleic acid. The diameter of the acne was significantly decreased in the nanocrystals group compared to the commercial formulation of azelaic acid [32]. Also, the nanocrystals group presented the lowest color difference between acne area and surrounding area. There was a recovery of the thickness of the epidermis to a healthy state when acne was treated with nanocrystals. The authors concluded that the azelaic acid nanocrystals presented a greater therapeutic effect on acne, mainly due to hair follicle-targeting and anti-inflammatory and antibacterial effects [32].
It is important to note that none of the selected articles evaluated antibiofilm activity of nanocrystals. However, biofilm formation in skin infections should be considered, as it can compromise treatment efficacy and contribute to therapeutic resistance [42]. This represents a relevant gap in literature and suggests the need for futures studies to investigate the potential of nanocrystals in disrupting established biofilms or preventing their formation.

6. Effect mechanism of nanocrystals in skin injections

In general, the microbiological tests conducted by the authors in this review confirmed the greater effectiveness of nanocrystal formulations over conventional systems, regarding skin infections. Although *in vitro* tests are interesting for comparing different substances or different delivery systems, it is important to mention that 7 articles (50%), were able to prove the higher efficacy of nanocrystals in animal models.
Nanocrystals present unique properties that may be associated with enhanced antimicrobial activity in skin infections, including increased bioadhesion, increased skin penetration, ability to penetrate hair follicles, increased saturation solubility and drug diffusion, and ability to penetrate microbial membranes (figure 4).
Several studies emphasize the reduced particle size as a key factor contributing to the increased activity [4, 20, 22, 24, 26, 27, 30–32]. Particle size reduction leads to an increase in surface area, promoting stronger interactions with microorganisms [17]. This greater contact area facilitates the adhesion of nanocrystals to the microbial surfaces, which may result in disruption of membrane integrity and interference with vital cellular functions Furthermore, improved solubility leads to increased drug diffusion and penetration through microbial membranes [43].
The bio-adhesive properties of nanocrystals lead to prolonged adhesion to biological surfaces and increase the drug concentration at the site of action, enhancing the contact time with microorganisms and contributing to a sustained antimicrobial activity [17]. Moreover, nanocrystals demon-

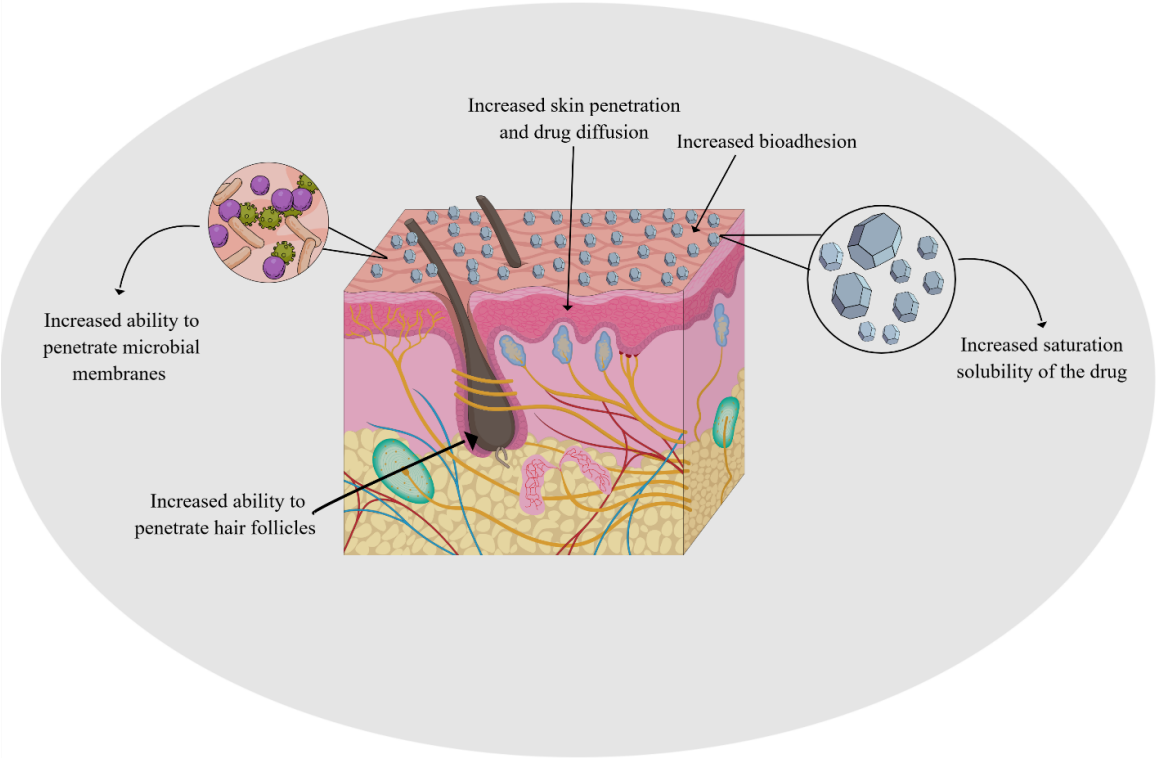

Figure 4. Nanocrystals properties that may be associated with enhanced antimicrobial activity in skin infections.

strate a superior ability to incorporate higher drug concentrations compared to other nanoparticles [16]. This results from the fact that, in nanocrystals, the drug itself forms the matrix of the system, requiring only the addition of a stabilizing agent, such as surfactants [16]. The increase in drug concentration may also contribute to enhanced antimicrobial activity, as higher concentrations promote a greater diffusion gradient [16], facilitating the penetration of the active compound in microbial membranes and thereby enhancing its action at the site of infection.

According to Parveen and co-workers (2023), topical nanocrystals can provide drug delivery for both instant release and controlled release [14]. Because of this, they hold great potential for use in topical treatments, as these nanoparticles are capable of modulating drug penetrating, for both deep and superficial skin layers [14, 17]. Nanocrystals can also enhance chemical and photostability of the drugs, which indirectly leads to higher efficacy, and to reduce systemic toxicity [14].

7. Safety considerations of nanocrystals

Six articles performed cytocompatibility tests, three of which used a fibroblast cell lineage [20, 24, 29] and three of which used other cell lineages [22, 30, 32]. In two articles, nanocrystals cytotoxicity was not observed [22, 29]. Anjani and co-workers (2022) evaluated the biocompatibility of metronidazole nanocrystals against fibroblast cells (3T3L1) using MTT assay and cell proliferation assays, and the authors conclude that the developed nanocrystals were biocompatible with minimal cytotoxicity [29]. In the same way, Omolo and co-workers evaluated the cytotoxicity of fusidic acid nanocrystals using an MTT assay, and the developed formulation showed 75% cell viability for epithelial cell line (A549) and human embryonic kidney cell (HEK 293) [22].

On the other hand, formulations containing sulfadiazine nanocrystals promoted lower cell viability than the coarse powder [20, 24]. The authors reported that this result might be due to the higher interaction between fibroblasts and silver sulfadiazine, induced by the high surface-to-volume ratio of nanocrystals. As particle size decreases, the surface-to-volume ratio increases, which enhances the reactivity of the nanocrystals and their interaction with biological systems. A higher surface area facilitates adhesion and penetration through cellular membranes, which may result in cellular damage and reduced viability [17] It is important to mention that nanocrystals ranging from 100 to 1000 nm are generally biodegradable and present lower toxicity [17]. In contrast, nanocrystals smaller than 100 nm may have the ability to enter cells through endocytosis mechanism, increasing their toxic potential and further reducing cell viability in *in vitro* models [17]. Furthermore, the higher concentration of dissolved drug enhanced its solubility [20, 24]. Trousil and co-workers (2022) used human skin epithelium A431 and human skin keratinocyte HaCaT cell lines and reported a risk of cytotoxicity with chloroxine nanocrystals. Nevertheless, it was argued that nanocrystals are not expected to interact directly with viable cells, because of the presence of the stratum corneum layer [30]. Macrophages (RAW 264.7) were evaluated regarding compatibility with azelaic acid nanocrystals, since the formulation was intended to have anti-inflammatory activity [32]. A decrease in cellular viability was only observed for the highest concentration of nanocrystals (0.5%) [32].

The skin irritation potential of the chloroxine nanocrystal formulation was tested *in vivo* on mice for 7 days, with no erythema formation. Histopathological examination of the skin revealed normal morphology, with absence of pathological processes in skin layers, skin appendages, and muscle tissue, indicating acceptable tolerance of chlorhexidine nanocrystals by mice [30]. Similarly, Kumar and co-workers (2019) performed an irritation test in albino Wistar rats to observe the irritation potential of luliconazole nanocrystal hydrogel. After 48 h, the formulation did not cause erythema, resulted in poor edema scores, and afforded a mean skin irritation score of 0.33 (slight redness in mouse). These findings demonstrate the non-irritating nature of the gel for human skin application [23]. Patel and co-workers (2019) conducted a skin irritation test in albino Wistar rats to evaluate the irritation potential of the clotrimazole nanogel [4]. The erythema score of the marketed formulation was higher, whereas the edema score of the clotrimazole nanogel was insignificant at all points. Such good results might have been due to the use of a stabilizing agent with minimal potential for irritation [4].

Ji and co-workers (2024) conducted a skin irritation test using abdominal skin of New Zealand white rabbits, in order to evaluate azelaic acid nanocrystals [32]. The optimized formulation with ultrasound did not show irritant reaction (erythema and edema), resulting in an irritation score of 0. In contrast, the commercial product produced mild erythema with an irritation score of around 0.2 [32]. These findings indicate that the developed nanocrystals formulation, when combined with ultrasound, offers improved skin safety compared to the commercial product [32].

Liu and co-workers (2023) used the modulated Karber method to determine the median lethal dose (LD_{50}) of a gel containing silver sulfadiazine nanocrystals [25]. The results confirmed the safety of the formulation, given that the LD_{50} was considerably higher (252.1 mg/kg) than the doses applied for *in vivo* burn wound treatment ($40 - 50$ mg/kg) [25]. Overall, the results show that nanocrystal formulations are compatible with skin applications.

8. Limitations and future perspective

The low number of articles found on the topic is a limitation of this review, however, this finding is in line with the fact that the use of drug nanocrystals in dermatological applications is relatively recent, being previously centered on oral drug administration [2]. Another limitation is the fact that 6 articles (almost half of the selected ones), performed only *in vitro* antimicrobial tests, directly applying the nanocrystals to bacteria or fungi in cultures. Antibiofilm activity was not evaluated in any of the articles. Regarding safety data, only 5 articles used animal models. No tests in humans were found for efficacy or safety evaluation.

The nanocrystals technology is considered a breakthrough technology for cutaneous application. The outcomes of the reviewed articles indicate that the drug nanocrystals are promising for skin infections treatment. The fact that drug nanocrystals are already on the market regarding oral and parenteral routes proves the feasibility of the technology. Future directions indicate for the necessity of more preclinical and clinical trials in this area, in order for cutaneous formulations containing antimicrobial drug nanocrystals to reach the population.

9. Conclusion

This literature review evaluated and discussed the main results of topical nanocrystal formulations for the treatment of skin infections caused by bacteria and fungi. Most of the active substances tested in the cited studies are commonly used for the treatment of skin infections. However, in the form of nanocrystals, the formulations achieve more effective penetration into skin layers, with enhanced antimicrobial activity and better performance in wound contraction or acne treatment. The safety of nanocrystal formulations for skin application is suggested. The limited number of publications on the topic, coupled with the positive results, suggests the existence of a vast field with potential for exploration. The use of drug nanocrystals is a promising strategy to improve the efficiency of topical treatments for skin infections.

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Authors contributions

Authors have contributed equally in preparing and writing the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors assert that they do not have any identifiable conflicting financial interests or personal relationships that might be perceived to influence the work presented in this paper.

References

[1] R. R. Watkins and M. Z. David. "Approach to the Patient with a Skin and Soft Tissue Infection.". *Infect Dis Clin North Am*, 35:1–48, 2021.
DOI: https://doi.org/10.1016/J.IDC.2020.10.011.

[2] P. K. Parmar, J. Wadhawan, and A. K. Bansal. "Pharmaceutical nanocrystals: A promising approach for improved topical drug delivery.". *Drug Discov Today*, 26:2329–49, 2021.
DOI: https://doi.org/10.1016/J.DRUDIS.2021.07.010.

[3] B. Singh, P. K. Upadhyay, and M. Kumar. "Nanocrystal for Dermatological Application: A Comprehensive Review.". *Curr Nanosci*, page 48–60, 2022.
DOI: https://doi.org/10.2174/1573413717666210121143038.

[4] V. Patel, O. P. Sharma, and T. A. Mehta. "Impact of Process Parameters on Particle Size Involved in Media Milling Technique Used for Preparing Clotrimazole Nanocrystals for the Management of Cutaneous Candidiasis.". *AAPS PharmSciTech*, 20(5):175, 2019.
DOI: https://doi.org/10.1208/S12249-019-1368-1.

[5] F. L. dos Santos, J. C. Zilles, A. U. Machado, M. S. Marques, B. S. da Costa, I. C. Kulkamp Guerreiro, et al. "Polymeric Nanocapsules Containing Ozonated Oil and Terbinafine Hydrochloride as a Potential Treatment Against Dermatophytes.". *AAPS PharmSciTech*, 24: 1–12, 2023.
DOI: https://doi.org/10.1208/s12249-023-02657-x.

[6] T. R. Arunraj, N. S. Rejinold, S. Mangalathillam, S. Saroj, R. Biswas, and R. Jayakumar. "Synthesis, characterization and biological activities of curcumin nanospheres.". *J Biomed Nanotechnol*, 10:238–50, 2014.
DOI: https://doi.org/10.1166/JBN.2014.1786.

[7] H. Lboutounne, J. F. Chaulet, C. Ploton, F. Falson, and F. Pirot. "Sustained *ex vivo* skin antiseptic activity of chlorhexidine in poly(ε-caprolactone) nanocapsule encapsulated form and as a digluconate.". *Journal of Controlled Release*, 82:319–34, 2002.
DOI: https://doi.org/10.1016/S0168-3659(02)00142-6.

[8] S. Jain, S. K. Jain, P. Khare, A. Gulbake, D. Bansal, and S. K. Jain. "Design and development of solid lipid nanoparticles for topical delivery of an anti-fungal agent.". *Drug Deliv*, 17:443–51, 2010.
DOI: https://doi.org/10.3109/10717544.2010.483252.

[9] F. Fernández-Campos, B. Clares Naveros, O. López Serrano, C. Alonso Merino, and A. C. Calpena Campmany. "Evaluation of novel nystatin nanoemulsion for skin candidosis infections.". *Mycoses*, 56:70–81, 2013.
DOI: https://doi.org/10.1111/J.1439-0507.2012.02202.X.

[10] S. Jacobus Berlitz, P. Reginatto, G. Machado, R. M. da, A. M. Fuentefria, F. D. P. Morisso, R. V. Contri, et al. "Development of a Clioquinol Nanocarrier as a New, Promising Option for the Treatment of Dermatomycosis. ". *Pharmaceutics*, 15(2):531, 2023.
DOI: https://doi.org/10.3390/PHARMACEUTICS15020531.

[11] Z. Rukavina, M. Šegvić Klarić, J. Filipović-Grčić, J. Lovrić, and Ž. Vanić. "Azithromycin-loaded liposomes for enhanced topical treatment of methicillin-resistant Staphyloccocus aureus (MRSA) infections.". *Int J Pharm*, 553(1-2):109–19, 2018.
DOI: https://doi.org/10.1016/J.IJPHARM.2018.10.024.

[12] B. Sadeghi and B. Koupaei. "Biogenic Synthesis of Silver Nanoparticles Using Fruit Aqueous Extract of Psidium Guajava and Its Antibacterial Activity.". *J Nanoanalysis*, 4:126–133, 2017.
DOI: https://doi.org/10.22034/jna.2017.02.005.

[13] M. Pourjafari, M. Ghane, H. Kaboosi, B. Sadeghi, and A. Rezaei. "Antibacterial Properties of Ag-Cu Alloy Nanoparticles Against Multidrug-Resistant Pseudomonas aeruginosa Through Inhibition of Quorum Sensing Pathway and Virulence-Related Genes.". *J Biomed Nanotechnol*, 18(4):1196–1204, 2022.
DOI: https://doi.org/10.1166/JBN.2022.3331.

[14] N. Parveen, M. A. S. Abourehab, P. V. Thanikachalam, R. K. Khar, and P. Kesharwani. "Nanocrystals as an emerging nanocarrier for the management of dermatological diseases.". *Colloids Surf B Biointerfaces*, 225:113231, 2023.
DOI: https://doi.org/10.1016/J.COLSURFB.2023.113231.

[15] M. B. McGuckin, J. Wang, R. Ghanma, N. Qin, S. D. Palma, R. F. Donnelly, et al. "Nanocrystals as a master key to deliver hydrophobic drugs via multiple administration routes.". *J Control Release*, 345: 334–53, 2022.
DOI: https://doi.org/10.1016/j.jconrel.2022.03.012.

[16] A. S. Alnaim. "Nanocrystals in Dermal Drug Delivery: A Breakthrough for Enhanced Skin Penetration and Targeted Skin Disorder Treatments.". *Pharmaceutics*, 16:1561, 2024.
DOI: https://doi.org/10.3390/PHARMACEUTICS16121561.

[17] V. Giradkar, A. Mhaske, and R. Shukla. "Nanocrystals: A Multifaceted Regimen for Dermatological Ailments.". *Particle & Particle Systems Characterization*, 41(6):2300147, 2024.
DOI: https://doi.org/10.1002/PPSC.202300147.

[18] R. H. Müller, X. Zhai, G. B. Romero, and C. M. Keck. "Nanocrystals for passive dermal penetration enhancement.". Springer, 2016.
DOI: https://doi.org/10.1007/978-3-662-47862-2_18/FIGURES/6.

[19] V. Singh, K. Bansal, H. Bhati, and M. Bajpai. "New Insights into Pharmaceutical Nanocrystals for the Improved Topical Delivery of Therapeutics in Various Skin Disorders.". *Curr Pharm Biotechnol*, 25:1182–1198, 2024.
DOI: https://doi.org/10.2174/0113892010276223231027075527.

[20] L. Gao, H. Gan, Z. Meng, R. Gu, Z. Wu, X. Zhu, et al. "Evaluation of genipin-crosslinked chitosan hydrogels as a potential carrier for silver sulfadiazine nanocrystals.". *Colloids Surf B Biointerfaces*, 148: 343–53, 2016.
DOI: https://doi.org/10.1016/j.colsurfb.2016.06.016.

[21] S. M. Pyo, D. Hespeler, C. M. Keck, and R. H. Müller. "Dermal miconazole nitrate nanocrystals - formulation development, increased antifungal efficacy & skin penetration.". *Int J Pharm*, 531(1):350–9, 2017.
DOI: https://doi.org/10.1016/j.ijpharm.2017.08.108.

[22] C. A. Omolo, R. S. Kalhapure, N. Agrawal, S. Rambharose, C. Mocktar, and T. Govender. "Formulation and Molecular Dynamics Simulations of a Fusidic Acid Nanosuspension for Simultaneously Enhancing Solubility and Antibacterial Activity.". *Mol Pharm*, 15:3512–26, 2018.
DOI: https://doi.org/10.1021/acs.molpharmaceut.8b00505.

[23] M. Kumar, N. Shanthi, A. K. Mahato, S. Soni, and P. S. Rajnikanth. "Preparation of luliconazole nanocrystals loaded hydrogel for improvement of dissolution and antifungal activity.". *Heliyon*, 5(5): e01688, 2019.
DOI: https://doi.org/10.1016/j.heliyon.2019.e01688.

[24] X. Liu, H. Gan, C. Hu, W. Sun, X. Zhu, Z. Meng, et al. "Silver sulfadiazine nanosuspension-loaded thermosensitive hydrogel as a topical antibacterial agent.". *Int J Nanomedicine*, 14:289–300, 2018.
DOI: https://doi.org/10.2147/ijn.s187918.

[25] X. Liu, H. Fan, Z. Meng, Z. Wu, R. Gu, X. Zhu, et al. "Combined Silver Sulfadiazine Nanosuspension with Thermosensitive Hydrogel: An Effective Antibacterial Treatment for Wound Healing in an Animal Model.". *Int J Nanomedicine*, 18:679–691, 2023.
DOI: https://doi.org/10.2147/ijn.s395004.

[26] I. S. Ahmed, O. S. Elnahas, N. H. Assar, A. M. Gad, and R. El. Hosary. "Nanocrystals of Fusidic Acid for Dual Enhancement of Dermal Delivery and Antibacterial Activity: *In Vitro*, *Ex Vivo* and *In Vivo* Evaluation.". *Pharmaceutics*, 12(3):199, 2020.
DOI: https://doi.org/10.3390/pharmaceutics12030199.

[27] A. D. Permana, A. J. Paredes, F. Volpe-Zanutto, Q. K. Anjani, E. Utomo, and R. F. Donnelly. "Dissolving microneedle-mediated dermal delivery of itraconazole nanocrystals for improved treatment of cutaneous candidiasis.". *European Journal of Pharmaceutics and Biopharmaceutics*, 154:50–61, 2020.
DOI: https://doi.org/10.1016/j.ejpb.2020.06.025.

[28] M. Rofeal, F. A. El-Malek, and X. Qi. "*in vitro* assessment of green polyhydroxybutyrate/chitosan blend loaded with kaempferol nanocrystals as a potential dressing for infected wounds.". *Nanotechnology*, 32(37), 2021.
DOI: https://doi.org/10.1088/1361-6528/abf7ee.

[29] Q. K. Anjani, A. H. Bin Sabri, J. Domínguez-Robles, N. Moreno-Castellanos, E. Utomo, L. A. H. Wardoyo, et al. "Metronidazole nanosuspension loaded dissolving microarray patches: An engineered composite pharmaceutical system for the treatment of skin and soft tissue infection.". *Biomaterials Advances*, 140:213073, 2022.
DOI: https://doi.org/10.1016/j.bioadv.2022.213073.

[30] J. Trousil, J. Matejková, Y. S. Dai, T. Urbánek, M. Šlouf, M. Škorič, et al. "Nanocrystalline chloroxine possesses broad-spectrum antimicrobial activities and excellent skin tolerability in mice.". *Appl Phys A: Mater Sci Process*, 17:1–14137–49, 2022.
DOI: https://doi.org/10.2217/nnm-2021-0323.

[31] M. B. Najm, M. Rawas-Qalaji, N. H. Assar, R. Yahia, R. El Hosary, and Ahmed I. S. "Optimization, characterization and in vivo evaluation of mupirocin nanocrystals for topical administration.". *European Journal of Pharmaceutical Sciences*, 176:106251, 2022.
DOI: https://doi.org/10.1016/j.ejps.2022.106251.

[32] Y. Ji, H. Li, J. Li, G. Yang, W. Zhang, Y. Shen, et al. "Hair Follicle-Targeted Delivery of Azelaic Acid Micro/Nanocrystals Promote the Treatment of Acne Vulgaris.". *Int J Nanomedicine*, 19:5173–91, 2024.
DOI: https://doi.org/10.2147/ijn.s459788.

[33] S. Verma, S. Kumar, R. Gokhale, and D. J. Burgess. "Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening.". *Int J Pharm*, 406(1-2):145–52, 2019.
DOI: https://doi.org/10.1016/j.ijpharm.2010.12.027.

[34] I. S. Mohammad, H. Hu, L. Yin, and W. He. "Drug nanocrystals: Fabrication methods and promising therapeutic applications.". *Int J Pharm*, 562:187–202, 2019.
DOI: https://doi.org/10.1016/j.ijpharm.2019.02.045.

[35] J. U. A. H. Junghanns and R. H. Müller. "Nanocrystal technology, drug delivery and clinical applications.". *Int J Nanomedicine*, 3(3): 295–309, 2008.
DOI: https://doi.org/10.2147/ijn.s595.

[36] G. B. Romero, C. M. Keck, and R. H. Müller. "Simple low-cost miniaturization approach for pharmaceutical nanocrystals production.". *Int J Pharm*, 501(1-2):236–244, 2016.
DOI: https://doi.org/10.1016/j.ijpharm.2015.11.047.

[37] N. O. Chung, M. K. Lee, and J. Lee. "Mechanism of freeze-drying drug nanosuspensions. ". *Int J Pharm*, 437(1-2):42–50, 2012.
DOI: https://doi.org/10.1016/j.ijpharm.2012.07.068.

[38] O. Pelikh, P. L. Stahr, J. Huang, M. Gerst, P. Scholz, H. Dietrich, et al. "Nanocrystals for improved dermal drug delivery.". *European Journal of Pharmaceutics and Biopharmaceutics*, 128:170–178, 2018.
DOI: https://doi.org/10.1016/j.ejpb.2018.04.020.

[39] S. R. Falsafi, H. Rostamabadi, E. Assadpour, and S. M. Jafari. "Morphology and microstructural analysis of bioactive-loaded micro/-nanocarriers via microscopy techniques; CLSM/SEM/TEM/AFM.". *Adv Colloid Interface Sci*, 280:102166, 2020.
DOI: https://doi.org/10.1016/j.cis.2020.102166.

[40] J. Hadgraft and M. E. Lane. "Skin permeation: The years of enlightenment.". *Int J Pharm*, 305(1-2):2–12, 2005.
DOI: https://doi.org/10.1016/j.ijpharm.2005.07.014.

[41] R. V. Contri, L. A. Fiel, N. Alnasif, A. R. Pohlmann, S. S. Guterres, and M. Schäfer-Korting. "Skin penetration and dermal tolerability of acrylic nanocapsules: Influence of the surface charge and a chitosan gel used as vehicle. ". *Int J Pharm*, 507(1-2):12–20, 2016.
DOI: https://doi.org/10.1016/j.ijpharm.2016.03.046.

[42] S. L. Percival, C. Emanuel, K. F. Cutting, and D. W. Williams. "Microbiology of the skin and the role of biofilms in infection.". *Int Wound J*, 9(1):14–32, 2012.
DOI: https://doi.org/10.1111/j.1742-481x.2011.00836.x.

[43] N. Seedat, R. S. Kalhapure, C. Mocktar, S. Vepuri, M. Jadhav, M. Soliman, et al. "Co-encapsulation of multi-lipids and polymers enhances the performance of vancomycin in lipid-polymer hybrid nanoparticles: In vitro and in silico studies.". *Mater Sci Eng C Mater Biol Appl*, 61:616–630, 2016.
DOI: https://doi.org/10.1016/j.msec.2015.12.053.