BIOSCIENCE JOURNAL

EFFECT OF GLYCEROL MONOLAURATE NANOCAPSULES ON *Streptococcus mutans* BIOFILM IN VITRO

Leonardo Quintana Soares LOPES[1,2], Rodrigo de Almeida VAUCHER[3], Janice Luehring GIONGO[3], Patrícia Kolling MARQUEZAN[2], Roberto Christ Vianna SANTOS[2]

[1]Biomedicine Undergraduate Program, Faculdade CNEC Santo Ângelo, Santo Ângelo, Rio Grande do Sul, Brazil.
[2]Laboratory of Oral Microbiology Research, Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil.
[3]Postgraduate Program in Biochemistry and Bioprospecting, Laboratory of Biochemistry Research and Molecular Biology of Microorganisms (LaPeBBiOM), Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil.

Corresponding author:
Leonardo Quintana Soares Lopes
leonardoquintanalopes@gmail.com

How to cite: LOPES, L.Q.S., et al. Effect of glycerol monolaurate nanocapsules on *Streptococcus mutans* biofilm in vitro. *Bioscience Journal*. 2024, **40**, e40050. https://doi.org/10.14393/BJ-v40n0a2024-72052

Abstract

This study aimed to prepare and apply nanocapsules containing glycerol monolaurate for eradicating *Streptococcus mutans* biofilms. The interfacial deposition method of the preformed polymer synthesized the nanocapsules characterized for mean diameter, polydispersity index, zeta potential, pH, and morphology by transmission electron microscopy. The microdilution method investigated antimicrobial activity. Crystal violet staining determined biomass quantification and the ability to inhibit biofilm formation. The study also measured exopolysaccharide production and the number of viable colonies. The characterization outcomes indicated acceptable values for the mean diameter 198.1 ± 2, a polydispersity index of 0.087 ± 0.018, a zeta potential of -21.30 ± 2.00 mV, a pH of 6.19 ± 0.12, and typical nanostructure morphology. The evaluations of minimum inhibitory and bactericidal concentrations of glycerol monolaurate (free and nanoencapsulated) revealed their ineffectiveness in inhibiting microorganisms. Only free glycerol monolaurate inhibits *S. mutans* growth with 125 µg/mL. Biomass, exopolysaccharide content, and viable colonies in the biofilm were analyzed to assess the compounds' ability to inhibit biofilm formation. The tested compounds did not significantly reduce the formed biofilm. Despite unfavorable outcomes of the formulated preparation, further experimentation with a new formulation is encouraged to explore alternative strategies and potential improvements.

Keywords: Biofilm. Dental caries. Nanocapsules. Periodontal disease. *Streptococcus mutans.*

1. Introduction

Biofilm-associated infections are a significant global health concern, contributing to approximately 80% of all human infections (Dutta et al. 2021). Biofilms consist of microbial communities that irreversibly adhere to biological and synthetic surfaces, including medical devices, implants, and tissues such as teeth and bones (Costerton et al. 1999). Treating these structures is particularly challenging due to their resistance to antimicrobial agents and the host immune system.

The oral cavity harbors a diverse microbiota, essential for maintaining oral health. However, shifting from a balanced microbiome to a pathogenic state may cause dental caries, gingivitis, and periodontitis, among other diseases (Lamont et al. 2018). *Streptococcus mutans* is a vital contributor to dental caries and

forms biofilms on tooth surfaces, metabolizing dietary sugars to produce acids that demineralize enamel, ultimately causing tooth decay (Bowen et al. 2018). Extracellular polysaccharides synthesized from sucrose facilitate biofilm formation by *S. mutans*, creating a matrix that enhances bacterial adherence and resilience (Koo et al. 2013).

Recent studies estimate that the human oral cavity hosts over 700 distinct microbial species, with mutans streptococci, particularly *S. mutans*, playing a central role in dental caries pathogenesis (Baker et al. 2024). The critical significance of controlling dental biofilms has driven research into novel antimicrobial agents that effectively target these communities without disrupting the overall balance of the oral microbiome (Kuang, Chen, and Xu 2018).

Glycerol monolaurate (GML), or monolaurin, is a glycerol monoester of lauric acid with known antimicrobial properties against various Gram-positive and Gram-negative bacteria and fungi (Schlievert et al. 2019). While GML is widely used as an emulsifier in the food and cosmetic industries, its potential as a systemic antimicrobial agent remains underexplored, primarily due to its limited water solubility and high melting point (Lopes et al. 2016).

Nanotechnology offers promising solutions to these limitations by enabling the development of nanostructured delivery systems that enhance the solubility, stability, and bioavailability of antimicrobial agents such as GML (Beyth et al. 2015). Nanocapsules, in particular, have a significant potential to deliver antimicrobial agents directly to biofilms, thereby improving their efficacy and minimizing adverse effects (Marzuoli et al. 2021). This study evaluated the effectiveness of nanocapsules containing glycerol monolaurate against oral pathogens such as *S. mutans*, considering their potential application in dental practice.

2. Material and Methods

Free and nanoencapsulated glycerol monolaurate

The development of the formulation containing glycerol monolaurate nanocapsules followed the interfacial deposition method of the preformed polymer (Fessi et al. 1989) with modifications. Polysorbate 80 (0.194 g) and ultrapure Milli-Q water (134 mL) were dissolved in the aqueous phase under moderate stirring at 40°C. In the organic phase, sorbitan monooleate (0.194 g), capric/caprylic acid triglycerides (0.8 g), a polymer blend of polymethyl methacrylate and polyethylene glycol (0.25g), and glycerol monolaurate (0.025 g) were dissolved in acetone (100%) under moderate agitation at 40°C. The aqueous phase then received the poured organic phase, and the mixture remained under moderate stirring for ten minutes. A rotary evaporator evaporated the solvent and water to adjust the concentration to 1 mg/ml. A blank formulation was prepared in the same way but without the glycerol monolaurate, and the free glycerol monolaurate was prepared by weighing 10 mg of the compound and solubilizing it in 10 mL of absolute ethanol.

Nanocapsule characterization

The produced formulations were characterized by their average diameter and polydispersity index using dynamic light scattering. Electrophoresis aided by a Zetasizer Nano-ZS (Malvern Instruments, United Kingdom) determined the zeta potential. A potentiometer (Digimed®) assessed the pH of the formulations. All parameter analyses occurred in three replicates.

The morphological analysis of glycerol monolaurate and blank nanocapsules used a transmission electron microscope operating at 80 kV (Transmission Electron Microscopy; Jeol, JEM 1200 Exll, Japan). The formulation was diluted (1:10 v/v in distilled water), and 1 mL was deposited on a grid (Formvar-Carbon support films), negatively stained with uranyl acetate solution (2% w/v), and observed at a magnification of 200.000 ×.

Microorganism

The strain of *Streptococcus mutans* was purchased from the American Type Culture Collection (ATCC 25175) for microbiological testing. The microorganism remained in a culture medium (Brain Heart Infusion - BHI) with glycerol and cooled to -80°C. The strain was thawed, inoculated on BHI broth, and incubated at 37°C for 24 hours to perform the tests.

Determination of minimum inhibitory and bactericidal concentrations

The broth microdilution method determined the minimum inhibitory concentration (MIC) in a 96-well plate (CLSI, 2015). All wells received the BHI broth (100 µL), and the first row received 100 µL of free or nanoencapsulated glycerol monolaurate, followed by serial dilution. Next, the inoculum (0.5 on the McFarland scale) containing *S. mutans* (15 µL) was added. The plate was incubated at 37°C for 24 hours. The well containing only the inoculum and broth was the positive control, and the negative control contained only the broth with saline. The test occurred in five replicates. After incubation, an addition of 25 µL of resazurin (0.015%) revealed the experiment. The MIC represented the lowest concentration inhibiting visible microbial growth. Regarding the minimum bactericidal concentration (MBC), 1 µL was collected from each well, seeded onto an agar plate with BHI, and incubated at 37°C for 24 hours. Colonies were identified, and the MBC represented the lowest concentration with undetected microbial growth (Russel and Furr 1977).

Biofilm formation

Biofilm production followed previous conditions (O'Toole et al. 1999) with modifications. Biofilm formation occurred by adding 15 µL of inoculum (0.5 on the McFarland scale) to flat-bottomed 96-well plates (Nunclon™ D surface, Nunc, Roskilde, Denmark) containing 100 µL of BHI broth, and the plate was incubated at 37°C for 24 hours. Subsequently, 100 µL of glycerol monolaurate or a glycerol monolaurate nanocapsule solution (500 µg/mL) was added, and the plate was incubated at 37°C for 24 hours. The *S. mutans* strain in BHI broth was the positive control, and BHI broth alone was the negative control.

Crystal violet assay

The described crystal violet technique quantified the biomass (Kouidhi et al. 2010). The supernatant was removed, and the well plate was washed three times with distilled water. The plate was incubated at 60°C for 60 minutes to fix the biofilm and stained with 150 µL of crystal violet (1%) for 15 minutes. The well plate was washed with distilled water to remove excess crystal violet. 150 µL of ethanol (95%) was added to dissolve the stain for ten minutes. Then, 100 µL were transferred to another plate to measure absorbance at 570 nm in a microplate reader (TP -Reader; ThermoPlate, Goiás, Brazil). The difference between the optical density of the positive control (BHI broth + *S. mutans*) and the compounds (glycerol monolaurate and glycerol monolaurate nanocapsules) determined anti-biofilm activity.

Inhibition of cell attachment

A microplate biofilm assay tested the anti-adhesion properties of glycerol monolaurate and glycerol monolaurate nanocapsules (Merritt et al. 2011). The well-plate containing BHI broth and glycerol monolaurate or glycerol monolaurate nanocapsules (500 µg/mL) received the inoculum (15 µL) containing *S. mutans*. The plate was incubated at 37°C for 24 hours. The assay occurred as described. BHI broth containing *S. mutans* was the positive control, and the inhibition percentage was calculated by $\left(\frac{Optical\ Density\ Test}{Optical\ Density\ Control}\right) \times 100.$

Quantification of biofilm cultivable cells

Colony-forming unit (CFU) counts determined the number of viable cells in the biofilm (Lopes et al. 2019). After adding the compounds, the wells were washed three times with phosphate buffer saline to remove non-adherent cells. The phosphate buffer saline (100 µL) was added, and the biofilm was scrapped with a tip. The cell suspension was shaken, diluted in phosphate buffer saline, plated on BHI agar, and incubated at 37°C for 24 hours. After incubation, the total CFU was counted, and the log CFU per milliliter (log_{10} CFU/mL) was determined.

Exopolysaccharide assay

The described method (Shetlar et al. 1948), adapted by Dall & Herndon (1989) with some modifications, determined the number of exopolysaccharides. The wells were washed three times with 0.9% NaCl. The phosphate buffer saline (100 µL) was added, and the plate was sonicated (two minutes, 60 W power). Samples were centrifuged (950 g for 15 minutes), and the supernatant was added to absolute ethanol (1 ml) and centrifuged again (2400 g for 20 minutes). The pellet was resuspended in distilled water (1 ml), mixed with sulfuric acid (77%, 7 ml), and placed in an ice bath for ten minutes. Tryptophan (1%, 1 ml) was added and mixed. The mixture was heated in a boiling water bath for 20 minutes, and absorbance was measured at 500 nm (TP -Reader; ThermoPlate, Goiás, Brazil). The control comprised only microorganisms and the medium.

Statistical analysis

Results of microbiological tests were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test with 95-99% significance ($p < 0.05$). All data were expressed as mean ± standard deviation. Experiments were performed in five replicates and three independent experiments.

3. Results

Nanocapsule characterization

After preparation, the glycerol monolaurate and blank nanocapsules underwent physicochemical characterization (Table 1). Morphological analysis by transmission electron microscopy revealed a characteristic nanometer size and spherical shape of nanocapsules. Figure 1 presents the image.

Table 1. Physicochemical characterization of nanoemulsions, evaluating size, polydispersity index (PDI) and zeta potential.

	Mean diameter (nm)	Polydispersity index	Zeta potential (mV)	pH
Glycerol Monolaurate Nanocapsules	198.1 ± 2	0.087 ± 0.018	-21.30 ± 2.00	6.19± 0.12
Blank Nanocapsules	186.5 ± 4.0	0.053 ± 0.015	-14.8 ± 6.1	6.28 ± 0.11

Minimum inhibitory and bactericidal concentrations

After adding the indicator reagent, the free glycerol monolaurate inhibited *S. mutans* growth with 125 µg/mL. The formulation containing glycerol monolaurate nanocapsules did not inhibit *S. mutans* growth. Both compounds did not eliminate *S. mutans* when determining the minimum bactericidal concentration. The nanocapsules were tested with sensitive microorganisms as a positive control. Our research group has published these tested microorganisms (Lopes et al. 2016; Lopes et al. 2016; Lopes et al. 2019).

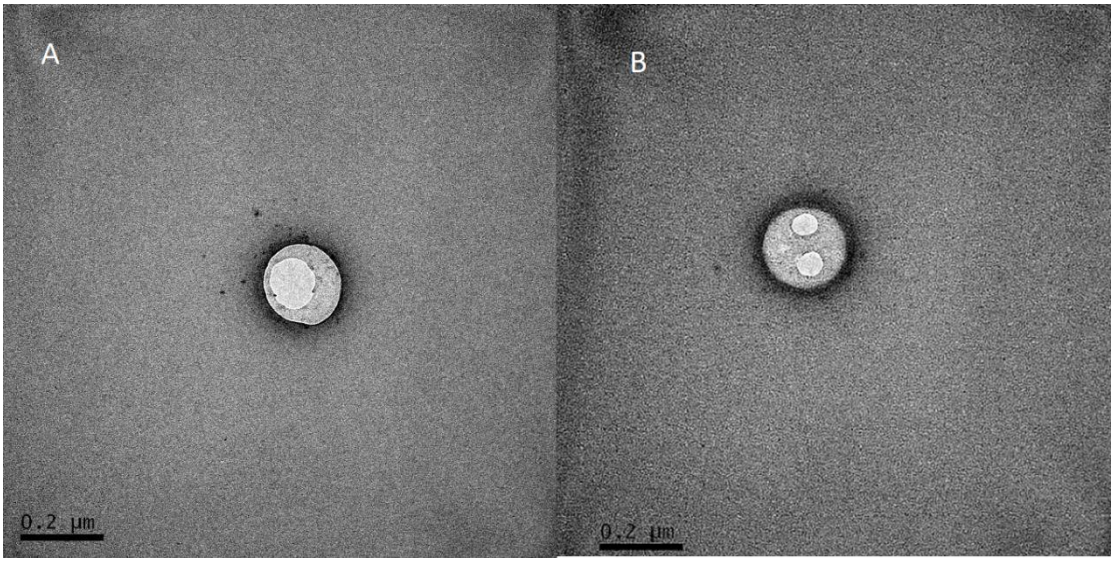

Figure 1. Transmission electron microscopy image of the PMMA/PEG glycerol monolaurate nanocapsule (A) and blank nanocapsule (B).

Anti-biofilm activity

Both compounds had no anti-biofilm effect. Free glycerol monolaurate and nanocapsules did not significantly reduce biomass, exopolysaccharides, and the number of viable cells in the biofilm. Also, the tested compounds did not inhibit *S. mutans* biofilm formation (Figure 2).

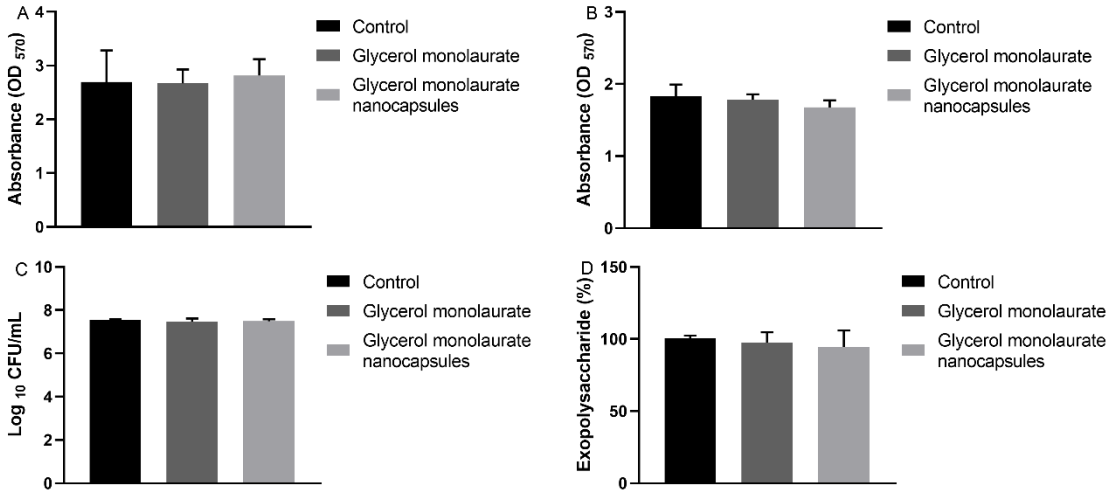

Figure 2. A - Biomass quantification by crystal violet staining; B - Biofilm inhibition efficiency of glycerol monolaurate and glycerol monolaurate nanocapsules; C - Effect of glycerol monolaurate and glycerol monolaurate nanocapsules on viable cells; D - Exopolysaccharide quantification on the biofilm treated with glycerol monolaurate and glycerol monolaurate nanocapsules. The control included only the medium with the microorganism. The data were expressed as mean ± standard deviation. The study applied a one-way analysis of variance (ANOVA) followed by Tukey's test at 95-99% significance ($p < 0.05$).

4. Discussion

Nanocapsule characterization outcomes agree with previous studies employing polymeric blends as drug carriers for nanoparticle development. Khalil et al. (2013) successfully developed curcumin nanoparticles using a PLGA-PEG blend, reaching a particle diameter of approximately 152.37 nm and a polydispersity index (PDI) of 0.077. Similarly, Seremeta et al. (2013) reported nanoparticle development with a PCL-Eudragit® RS 100 blend, achieving a diameter of approximately 173 nm and a PDI of 0.077.

Dental biofilm remains the primary etiologic factor in dental caries and periodontal disease onset (Kuboniwa and Lamont 2010; Pitts et al. 2017). When improperly managed, biofilms may undergo

increased physiological heterogeneity and engage in complex interactions that accelerate tooth demineralization and systemic inflammation (Pitts et al. 2017). Among critical bacterial species implicated in oral diseases is *S. mutans*, a Gram-positive bacterium known for its acidogenic and aciduric properties (Fejerskov et al. 2015; Bedoya-Correa et al. 2021).

Our study found that glycerol monolaurate (monolaurin) exhibited limited antimicrobial activity against *S. mutans*. Although we hypothesized that nanocapsule formulation would enhance monolaurin penetration into deeper biofilm layers, it did not perform as effectively as free monolaurin (Wu et al. 2021). This observation aligns with findings in the literature, in which monolaurin showed variable efficacy against different bacterial species. Schlievert and Peterson (2012) reported that monolaurin's effectiveness against Gram-negative bacteria, such as *Escherichia coli*, is highly pH-dependent and had significantly enhanced activity at lower pH levels.

Studies have explored the potential of monolaurin to combat *S. mutans* specifically. One study showed that monolaurin significantly inhibited *S. mutans* biofilm formation at concentrations as low as 24 mg/L, indicating its potential to disrupt biofilm development, a critical factor in dental caries progression (Ham and Kim 2016). Furthermore, research has highlighted the synergistic effects of monolaurin combined with traditional antibiotics, suggesting that this approach might enhance the overall antimicrobial efficacy against Gram positive bacteria within biofilms (Ghany et al. 2024).

While glycerol monolaurate nanocapsules alone may not sufficiently combat *S. mutans*, there is potential for optimizing the formulation or combining it with other antimicrobial agents to improve efficacy. The observed pH-dependent activity also emphasizes the need for further investigation into environmental conditions under which monolaurin is most effective, particularly in the dynamic and variable environment of the oral cavity. Future studies should explore these variables to develop more targeted and effective strategies for preventing and managing oral diseases related to *S. mutans*.

5. Conclusions

The study successfully prepared the nanocapsule containing glycerol monolaurate with good characterization values. However, the free and encapsulated compounds showed no potential against *S. mutans* in free and sessile forms. Hence, the compounds may be applied as therapy to combat the biofilm formed by this microorganism. Further studies with different nanostructures should search for viable and safe alternatives of nanotechnology applications to oral biofilms.

Authors' Contributions: LOPES, L.Q.S.: conceptualization, methodology, formal analysis, writing - original draft, investigation, data curation, visualization, project administration, funding acquisition; MARQUEZAN, P.K.: investigation, validation, formal analysis, writing – review & editing. VAUCHER, R.A.: resource, data curation, writing – review & editing, supervision. GIONGO, J.L.: resource, data curation, writing – review & editing, supervision. SANTOS, R.C.V.: conceptualization, writing – review & editing, resource, supervision.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: This work received financial support of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) [Process number: 151863/2020-2], and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

BAKER, J. L., et al. The oral microbiome: diversity, biogeography and human health. *Nature Reviews Microbiology*, 2024, **22**, 89–104. https://doi.org/10.1038/s41579-023-00963-6

BEDOYA-CORREA, C. M., RINCÓN-RODRÍGUEZ, R. J., & PARADA-SANCHEZ, M. T. Acidogenic and aciduric properties of Streptococcus mutans serotype c according to its genomic variability. *European Journal of Oral Sciences*, 2021, **129**(6), e12824. https://doi.org/10.1111/eos.12824

BEYTH, N., HOURI-HADDAD, Y., DOMB, A., KHAN, W., & HAZAN, R. Alternative antimicrobial approach: nano-antimicrobial materials. *Evidence-Based Complementary and Alternative Medicine*, 2015, **2015**, 246012. https://doi.org/10.1155/2015/246012

BOWEN, W. H., & KOO, H. Biology of Streptococcus mutans-derived glucosyltransferases: Role in extracellular matrix assembly and biofilm formation. *Caries Research*, 2018, **52**(5), 291-307. https://doi.org/10.1159/000324598

CHOPRA, H., et al. Curcumin nanoparticles as promising therapeutic agents for drug targets. *Molecules (Basel, Switzerland)*, 2021, **26**(16), 4998. https://doi.org/10.3390/molecules26164998

CLSI, Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard — Ninth Edition. *CLSI document M07-A9*, 2015, **32**(2).

COSTERTON, J. W., STEWART, P. S., & GREENBERG, E. P. Bacterial biofilms: A common cause of persistent infections. *Science*, 1999, **284**(5418), 1318-1322. https://doi.org/10.1126/science.284.5418.1318

DALL, L., & HERNDON, B. Quantitative assay of glycocalyx produced by viridans group streptococci that cause endocarditis. *Journal of clinical microbiology*,1989, **27**(9), 2039–2041. https://doi.org/10.1128/jcm.27.9.2039-2041.1989

DUTTA, B., LAHIRI, D., NAG, M., MUKHERJEE, D., & RAY, R. R. Introduction to bacterial biofilm and acute infections. In: RAY, R. R., NAG, M., & LAHIRI, D. (eds) *Biofilm-Mediated Diseases: Causes and Controls*. Springer, Singapore, 2021. https://doi.org/10.1007/978-981-16-0745-5_1

FEJERSKOV, O., et al. Dental caries: The disease and its clinical management. 3rd Edition. Wiley-Blackwell, 2015.

FESSI, H., et al. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics*, 1989, **55**(1), R1–R4. http://doi.org/10.1016/0378-5173(89)90281-0

GHANY, S. S. H. A. E., IBRAHEM, R. A., EL-GENDY, A. O., et al. Novel synergistic interactions between monolaurin, a mono-acyl glycerol, and β-lactam antibiotics against Staphylococcus aureus: an in vitro study. *BMC Infectious Diseases*, 2024, **24**, 379. https://doi.org/10.1186/s12879-024-09261-9

HAM, Y., & KIM, T. J. Inhibitory activity of monoacylglycerols on biofilm formation in Aeromonas hydrophila, Streptococcus mutans, Xanthomonas oryzae, and Yersinia enterocolitica. *SpringerPlus*, 2016, **5**, 1526. https://doi.org/10.1186/s40064-016-3182-5

KHALIL, N. M., et al. Pharmacokinetics of curcumin-loaded PLGA and PLGA–PEG blend nanoparticles after oral administration in rats. *Colloids and Surfaces B: Biointerfaces*, 2013, **101**, 353-360. https://doi.org/10.1016/j.colsurfb.2012.06.024

KOO, H., FALSETTA, M. L., & KLEIN, M. I. The exopolysaccharide matrix: A virulence determinant of cariogenic biofilm. *Journal of Dental Research*, 2013, **92**(12), 1065-1073. https://doi.org/10.1177/0022034513504218

KOUIDHI, B., ZMANTAR, T., & BAKHROUF, A. Anti-cariogenic and anti-biofilms activity of Tunisian propolis extract and its potential protective effect against cancer cells proliferation. *Anaerobe*, 2010, **16**, 566–571. http://doi.org/10.1016/j.anaerobe.2010.09.005

KUANG, X., CHEN, V., & XU, X. Novel approaches to the control of oral microbial biofilms. *BioMed Research International*, 2018, **6498932**. https://doi.org/10.1155/2018/6498932

KUBONIWA, M., & LAMONT, R. J. Subgingival biofilm formation. *Periodontology 2000*, 2010, **52**(1), 38-52. https://doi.org/10.1111/j.1600-0757.2009.00311.x

LAMONT, R. J., KOO, H., & HAJISHENGALLIS, G. The oral microbiota: Dynamic communities and host interactions. *Nature Reviews Microbiology*, 2018, **16**(12), 745-759. https://doi.org/10.1038/s41579-018-0089-x

LOPES, L. Q. S., et al. Characterisation and anti-biofilm activity of glycerol monolaurate nanocapsules against Pseudomonas aeruginosa. *Microbial Pathogenesis*, 2019, **130**, 178–185. http://doi.org/10.1016/j.micpath.2019.03.007

LOPES, L. Q. S., et al. Evaluation of antimicrobial activity of glycerol monolaurate nanocapsules against American foulbrood disease agent and toxicity on bees. *Microbial Pathogenesis*, 2016, **97**, 183–188. http://doi.org/10.1016/j.micpath.2016.05.014

LOPES, L. Q. S., et al. Nanocapsules with glycerol monolaurate: Effects on Candida albicans biofilms. *Microbial Pathogenesis*, 2016, **97**, 119–124. http://doi.org/10.1016/j.micpath.2016.05.016

MARZUOLI, I., et al.. Nanocapsule designs for antimicrobial resistance. *Nanoscale*, 2021, **13**(23), 10342–10355. https://doi.org/10.1039/d0nr08146a

MERRITT, J. H., KADOURI, D. E., & O'TOOLE, G. A. Growing and analyzing static biofilms. *Current Protocols in Microbiology*, 2011, No. SUPPL. 22. http://doi.org/10.1002/9780471729259.mc01b01s22

O'TOOLE, G. A., et al. Genetic approaches to study of biofilms. *Methods in Enzymology*, 1999, **310**, 91–109. http://doi.org/10.1016/S0076-6879(99)10008-9

PITTS, N. B., et al. Dental caries. *Nature Reviews Disease Primers*, 2017, **3**, 17030. https://doi.org/10.1038/nrdp.2017.30

RUSSEL, A. D., & FURR, J. R. The antibacterial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid. *Journal of Applied Bacteriology*, 1977, **43**(2), 253–260. http://doi.org/10.1111/j.1365-2672.1977.tb00749.x

SCHLIEVERT, P. M., & PETERSON, M. L. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. *PLoS One*, 2012, **7**(7), e40350. https://doi.org/10.1371/journal.pone.0040350

SCHLIEVERT, P. M., KILGORE, S. H., SEO, K. S., et al. Glycerol monolaurate contributes to the antimicrobial and anti-inflammatory activity of human milk. *Scientific Reports*, 2019, **9**, 14550. https://doi.org/10.1038/s41598-019-51130-y

SEREMETA, K. P., CHIAPPETTA, D. A., & SOSNIK, A. Poly(ε-caprolactone), Eudragit® RS 100 and poly(ε-caprolactone)/Eudragit® RS 100 blend submicron particles for the sustained release of the antiretroviral efavirenz. *Colloids and Surfaces B: Biointerfaces*, 2013, **102**, 441-449. https://doi.org/10.1016/j.colsurfb.2012.06.038

SHETLAR, M. R., FOSTER, J. V., & EVERETT, M. R. Determination of serum polysaccharides by the tryptophane reaction. *Proceedings of the Society for Experimental Biology and Medicine*, 1948, **67**(2), 125–130. http://doi.org/10.3181/00379727-67-16223

WU, S., et al. Biofilm-Sensitive Photodynamic Nanoparticles for Enhanced Penetration and Antibacterial Efficiency. *Advanced Functional Materials*, 2021, **31**, 2103591. https://doi.org/10.1002/adfm.202103591

Received: 11 January 2024 | **Accepted:** 20 August 2024 | **Published:** 30 October 2024