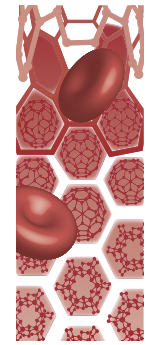
Commentary

For reprint orders, please contact: reprints@futuremedicine.com

Nanomedicine



Can dermal delivery of therapeutics be improved using thermoresponsive nanogels?

Neha Tiwari[1], Ana S Sonzogni[2] & Marcelo Calderón*[,1,3]

[1]POLYMAT & Applied Chemistry Department, Faculty of Chemistry, University of the Basque Country UPV/EHU, Paseo Manuel de Lardizabal 3, 20018, Donostia-San Sebastián, Spain

[2]Group of Polymers & Polymerization Reactors, INTEC (Universidad Nacional del Litoral-CONICET), Güemes 3450, Santa Fe 3000, Argentina

[3]IKERBASQUE, Basque Foundation for Science, 48013 Bilbao, Spain

*Author for correspondence: marcelo.calderon@polymat.eu

"thermoresponsive nanogels that undergo transition from swollen to collapsed state are capable of releasing loaded active molecules selectively past stratum corneum"

First draft submitted: 20 September 2019; Accepted for publication: 17 October 2019; Published online: 22 November 2019

Keywords: dermal drug delivery • hydration • nanocarriers • skin penetration • stratum corneum • temperature • thermoresponsive nanogels

Skin, being the largest organ of the body, has attracted a lot of attention in recent years as a vector to deliver a wide spectrum of cargo molecules to treat multiple conditions, including genetic disorders, infections by pathogens (bacteria, virus, fungus), inflammatory diseases such as psoriasis and atopic dermatitis, and skin cancer. In order to deliver active molecules across the skin layers, it is crucial to understand the morphology and properties of skin. A healthy skin is associated with a highly efficient barrier that prevents invasion of foreign particles or microbes from the external environment. As a consequence, the outermost layer of the epidermis, also called the stratum corneum (SC), prevents penetration of molecules that are larger than 500 Da [1]. This represents an immense challenge for delivery of bigger active molecules into the skin tissues via passive diffusion. Various formulations such as creams, gels and ointments have been studied to overcome the skin protective barrier but they mainly intend to have local effect rather than systemic action. To enhance penetration of active therapeutics across the skin, several techniques has been developed. This includes chemicals such as surfactants, alcohols, amines – among others, or physical disruption of the SC using methods such as sonoporation, iontophoresis, electroporation and microneedles [2]. Although penetration enhancers have proven to be effective for delivery of active therapeutics, they could lead to long-term or irreparable damage of the lipid structure of the SC. Nanogels, being cross-linked polymers with nanometer dimensions, provide an alternative approach to existing technologies with minimal damage to the natural barrier function of the skin. Furthermore, nanogels possess certain desirable features such as solubility and stabilization of hydrophobic drugs or proteins and the ability to target encapsulated moieties to specific cell types, with control over release profiles. In addition, nanogels that respond to various stimuli such as pH and temperature are shown to enhance the penetration of cargo molecules in the skin by interacting with the SC, followed by the triggered release of cargo molecules [3–5].

Stimuli sensitive nanogels in dermal drug delivery

Environmentally responsive nanogels have emerged as promising materials for biomedical applications due to their ability to respond to external stimuli for tissue-specific targeting or controlled drug release. In these systems, small changes in the physiological variables, such as temperature, pH or ionic strength, lead to reversible changes in nanogel's physicochemical properties, such as water content, volume, hydrophilicity–hydrophobicity – among others. In topical applications, responsive nanogels designed to exhibit a transition in natural conditions of skin, like native temperature and pH gradient along the different layers, could lead to penetration of the nanogels followed by release and deeper penetration of cargo molecules. Various pH and temperature (or a combination of both) responsive nanogels have been explored in the past to target various skin diseases. For example, Iyer

Future Medicine

et al. demonstrated the potential of biodegradable and pH-responsive chitosan [6] and double walled poly(lactic-co-glycolic acid)-chitosan [7] nanogels loaded with 5-fluorouracil to treat skin melanoma. The pH responsive behavior of the nanogels led to triggered release of 5-fluorouracil in acidic environment of the tumor site. In other studies, chitin-based nanogels have been explored to target inflammatory diseases such as psoriasis wherein deeper penetration of drug molecules is required. Chitin nanogels loaded with two antipsoriatic drugs, in other words, acitretin and aloe-emodin, demonstrated enhanced swelling and release at higher pH, with higher deposition of the nanogels at epidermal and dermal layers, as confirmed from skin permeation studies on porcine skin [8]. In addition, dual responsive nanogels of poly(N- isopropylacrylamide-co-acrylic acid) exhibiting both temperature and pH response were investigated by Heard *et al.* Here, nanogels enhanced the permeation of loaded caffeine by a magnitude of 3.5 in comparison to saturated solution of caffeine, indicating strong role of stimuli response in dermal drug delivery [9].

Promising features of thermoresponsive nanogels

Temperature induced release of therapeutics is among the most investigated strategies in biomedicine, given the wide range of applications where temperature variations are naturally present. Temperature triggers can be exploited using thermoresponsive nanogels (tNGs) that undergo a reversible volume change upon crossing the volume phase transition temperature, enabling the controlled release of encapsulated cargo. As normal skin has a temperature gradient between 32 and 37°C, along the SC, thermoresponsive nanogels that undergo transition from swollen to collapsed state are capable of releasing loaded active molecules selectively past stratum corneum, where temperature is above volume phase transition temperature of nanogels [10]. As an example, Elmazar *et al.* combined smart gold nanoparticles (AuNPs) and tNGs toward antibacterial and wound healing applications. The tNG formulations prepared using Pluronic 127 and hydroxypropyl methyl cellulose with AuNPs exhibited prolonged and sustained effects compared with AuNP suspension along with improved bioavailability, skin permeation, antibacterial and anti-inflammatory activity [11].

Extensive studies have been carried out in our group for skin penetration using nanogels principally based on dendritic polyglycerol (dPG) as macro cross-linker and different thermoresponsive polymers [12,13]. As a proof of concept, Hedtrich *et al.* reported TG1 loaded tNGs of dPG and poly(N-isopropylacrylamide) (dPG-PNIPAM) exhibiting thermal trigger at 35°C. In response to temperature, the nanogels show 20% reduction in size followed by 93% release of loaded proteins in TG1 deficient skin models. The TG1 delivery restored the barrier function of the skin model, which was lost, as TG1 deficiency results in disruption of normal keratinization of the skin. Such results are of particular importance for the treatment of autosomal recessive congenital ichthyosis, for which there are currently no curative therapies available [4,14]. In another study, tNGs encapsulated with the anti-TNF-α fusion protein etanercept (MW ~150 kDa) showed temperature responsive release of the protein in barrier-deficient skin models. Notably, it is one of the first reported strategies showing promising local anti-inflammatory activity in skin with topical application of proteins [5].

The application of dye-labeled tNGs on skin models, showed for the first time a temperature dependent interaction of the soft nanogels with skin barrier and hair follicles [15]. One of the probable explanations of the penetration of tNGs in skin is hydration of SC. In the studies, three different thermoresponsive polymers, namely, pNIPAM, poly(di(ethylene glycol) methyl ether methacrylate-co-oligo ethylene glycolmethacrylate) (DEGMA-co-OEGMA475), and poly(glycidyl methyl ether-co-ethyl glycidyl ether) (tPG) were used as linear counterparts and dPG was used as a macro cross-linker to fabricate nanogels. An increment in the penetration of tNGs and encapsulated fluorescein was detected using fluorescent microscopy on excised skin models wherein tNGs were found to disrupt the lipid and protein arrangement in the skin barrier due to the hydration effect of tNGs [16]. In follow-up studies, Rancan *et al.* successfully demonstrated the penetration of tNGs in the SC of both intact and disrupted skin models. The studies showed enhanced penetration of tNGs with a release of dye in the epidermis on thermal trigger by infrared radiation. Additionally, in barrier disrupted skin, considerable quantities of the tNGs and fluorochrome were detected in both epidermis and dermis and thus shows promising applications for the treatment of inflammatory skin diseases [17]. Similarly, two nanogels synthesized from dPG and tPG or pNIPAM as the thermoresponsive polymers are shown to have capability to incorporate high doses of dexamethasone (DXM) and tacrolimus for treatment of severe skin diseases [18,19]. Laser scanning confocal microscopy revealed successful localization of tNGs predominantly in lysosomal compartments. Toxicological studies carried out in this work showed that nanogels are well tolerated and could have potential for clinical trials. Additionally, studies conferring the interactions of tNGs with dendritic cells displayed no cytotoxic or genotoxic effects, or induction of

reactive oxygen species (ROS) in the cells, further indicating their potential [20]. In another study, beta cyclodextrin utilized as a natural penetration enhancer has been combined with dPG and linear tPG to obtain tNGs. *Ex vivo* experiments demonstrated efficient delivery of DXM to the epidermis and dermis of human skin. Furthermore, DXM loaded tNGs outperformed the commercially available DXM formulation and downregulated the expression of proinflammatory thymic stromal lymphopoietin [21]. Studies conducted on *ex vivo* pig ear skin have also highlighted the potential of tNGs in transfollicular drug delivery (hair follicles acts as reservoirs). The follicular penetration of tNGs was found to be proportional to the size of the nanogels. Also, nanogels undergo deeper follicular penetration above their cloud point temperature (T_{cp}) as a consequence of structural changes in their matrix [22,23]. In addition to drug and protein delivery for therapeutic purposes, tNGs are shown to have potential for transdermal vaccination. Sonzogni *et al.* compared the efficiency of three poly(N-vinylcaprolactam) (PVCL)-based thermoresponsive assemblies, in other words, hydrogels, nanogels and film forming PVCL/acrylic nanogels. The authors observed that PVCL nanogels enhance the penetration of topically applied ovalbumin, as antigen model protein, into *ex vivo* human skin as compared with the protein alone and to the protein released by the hydrogel. The nanogels achieved an effective delivery of protein into the viable epidermis, where immune cells are present [24].

Although temperature is the most explored trigger for drug delivery applications, it should be noted that up to date there are no nanocarriers that are used for clinical practices. Research conducted in our group shows promising results of tNGs for both small molecules and protein skin delivery which shows immense potential for advanced clinical trials.

Toxicology assessment as bottle neck of clinical development

With advancement in research of nanomaterials for biomedical applications, nanotoxicology has become crucial to understand the physicochemical interactions of nanomaterials with different cells and tissues to undergo bench to market translation. As highlighted in this commentary, tNGs have enormous potential for the topical delivery of therapeutics for treatment of severe skin diseases. Nevertheless, nanogels are prone to be captured by keratinocytes on crossing the SC, and thus their potential adverse effects must be elucidated before realization to clinical trials. Biocompatibility evaluation of tNGs for topical applications mainly use conventional assays like MTT. However, to fully assess the toxic effects of nanoparticles, further complementary tests are needed to be carried out. Kleuser *et al.* have reported various assays to determine the mechanism by which an exogenous agent can harm the cells. For instance, nanoparticles have the potential to generate intracellular ROS and genotoxic effects (commonly shown by the silver nanoparticles) reflected by the induction of DNA damage. Carboxy-H2DCFDA and comet assay on human keratinocyte cells can indicate the role of nanogels in generating ROS or DNA damage respectively. The negative observations on the mentioned assays can ensure the nontoxicity of nanogels on primary human keratinocytes. Furthermore, the potential irritation of nanogels can be tested on red blood cells and by performing bovine corneal opacity and permeability tests (BCOP). Negligible haemolytic activity of nanogels on red blood cells and lower irritation score from BCOP can confirm low irritability of the nanogels on application [19]. Although there are no reports claiming the transdermal penetration of nanogels, their potential systemic toxicity should also be considered. To determine toxicity of nanomaterials in *in vivo* environment, the Organization for Economic Cooperation and Development has established certain guidelines for oral toxicity, eye irritation, corrosion, dermal toxicity and LD_{50} in mice that must be fulfilled before human trials [25]. Although present *in vitro* and *in vivo* toxicity studies can help to understand the short term toxicity with wide variations in chemical nature of the nanomaterials, appropriate analysis and systematically designed experiments are needed for much deeper understanding of their interactions and prolonged toxic effects.

Future perspective

According to US FDA reports, the majority of the nanomaterials based formulations are limited to intravenous (59%) and oral administration (21%) and only 4% are used for topical delivery of drugs [26]. Research reflects that nanoparticles are promising candidates for dermal drug delivery, however, material toxicity and tissue selectivity are major hurdles for their successful translation to clinical use. Solid nanoparticles such as gold, silver, quantum dots – among others, show enhanced penetration and deeper delivery of drugs, nevertheless, they are prone to accumulate in the secondary organs, which could lead to prolonged toxic effects [27]. tNGs on the other hand, have proven to be interesting candidates as they can be developed taking advantage of natural malfunctions of the diseased or inflamed skin conditions with minimal damage to SC. However, detailed toxicological studies are

10.2217/nnm-2019-0345

needed to be carried out before they can be realized for clinical trials. Other major barriers in dermal drug delivery are associated with skin models that are currently used for research purposes: pig skin, mice skin, reconstructed skin models and *ex vivo* human skin. Although the mentioned models are appropriate to study the preliminary effects of nanocarriers in *in vitro,* they fail to mimic natural skin conditions due to absence of real complexity of the skin. In view of this, intensive research must be focused on designing skin models that can mimic the complex nature of different layers of skin and hair follicles. As an alternative, the validation of methods that make complementary use of the existing models should be considered. Taken together we may conclude that the current literature shows very interesting results for tNGs in dermal delivery of small molecules and proteins. In our opinion, the future of these systems must be focused on developing smart nanogels for delivery of peptides, proteins or genetic material to specific cell populations to cure severe diseases. Special emphasis must be given on the translation of nanogels for clinical purposes to merge the gap between research and the market as per FDA (and other agencies) guidelines.

Author contributions

All the authors conceived the outline and concept of the manuscript, and discussed the included references. N Tiwari drafted the first version of the manuscript. All authors commented, introduced corrections and gave approval to the final version of the manuscript.

Financial & competing interests disclosure

M Calderón is grateful to IKERBASQUE- Basque Foundation for Science for financial support. M Calderón and N Tiwari acknowledge Polymat, Basque Centre for Macromolecular Design and Engineering for funding. A Sonzogni acknowledges financial support from CONICET, Universidad Nacional del Litoral and the European Union Commission through RISE Horizon 2020, project 823989 IONBIKE. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp. Dermatol.* 9, 165–169 (2000).
2. Ting WW, Vest CD, Sontheimer RD. Review of traditional and novel modalities that enhance the permeability of local therapeutics across the stratum corneum. *Int. J. Dermatol.* 43(7), 538–547 (2004).
3. Sun H, Zielinska K, Resmini M, Zarbakhsh A. Interactions of NIPAM nanogels with model lipid multi-bilayers: a neutron reflectivity study. *J. Colloid Interface Sci.* 536, 598–608 (2019).
4 . Witting M, Molina M, Obst K *et al.* Thermosensitive dendritic polyglycerol-based nanogels for cutaneous delivery of biomacromolecules. *Nanomedicine* 11(5), 1179–1187 (2015).
5 . Giulbudagian M, Yealland G, Honzke S *et al.* Breaking the barrier – potent anti-inflammatory activity following efficient topical delivery of etanercept using thermoresponsive nanogels. *Theranostics* 8(2), 450–463 (2018).
6. Sahu P, Kashaw SK, Sau S *et al.* pH responsive 5-fluorouracil loaded biocompatible nanogels for topical chemotherapy of aggressive melanoma. *Colloids Surf. B Biointerfaces* 174, 232–245 (2019).
7. Sahu P, Kashaw SK, Jain S, Sau S, Iyer AK. Assessment of penetration potential of pH responsive double walled biodegradable nanogels coated with eucalyptus oil for the controlled delivery of 5-fluorouracil: *in vitro* and *ex vivo* studies. *J. Control. Release* 253, 122–136 (2017).
8. Divya G, Panonnummal R, Gupta S, Jayakumar R, Sabitha M. Acitretin and aloe-emodin loaded chitin nanogel for the treatment of psoriasis. *Eur. J. Pharm. Biopharm.* 107, 97–109 (2016).
9. Abu Samah NH, Heard CM. Enhanced *in vitro* transdermal delivery of caffeine using a temperature- and pH-sensitive nanogel, poly(NIPAM-co-AAc). *Int. J. Pharm.* 453(2), 630–640 (2013).
10. Vogt A, Wischke C, Neffe AT, Ma N, Alexiev U, Lendlein A. Nanocarriers for drug delivery into and through the skin – do existing technologies match clinical challenges? *J. Control. Release* 242, 3–15 (2016).
11. Arafa MG, El-Kased RF, Elmazar MM. Thermoresponsive gels containing gold nanoparticles as smart antibacterial and wound healing agents. *Sci. Rep.* 8(1), 13674 (2018).
12. Theune LE, Charbaji R, Kar M, Wedepohl S, Hedtrich S, Calderon M. Critical parameters for the controlled synthesis of nanogels suitable for temperature-triggered protein delivery. *Mater. Sci. Eng. C Mater. Biol. Appl.* 100, 141–151 (2019).
13. Rancan F, Asadian-Birjand M, Dogan S *et al.* Effects of thermoresponsivity and softness on skin penetration and cellular uptake of polyglycerol-based nanogels. *J. Control. Release* 228, 159–169 (2016).

14. Plank R, Yealland G, Miceli E *et al.* Transglutaminase 1 replacement therapy successfully mitigates the autosomal recessive congenital ichthyosis phenotype in full-thickness skin disease equivalents. *J. Invest. Dermatol.* 139(5), 1191–1195 (2019).
15. Asadian-Birjand M, Bergueiro J, Rancan F *et al.* Engineering thermoresponsive polyether-based nanogels for temperature dependent skin penetration. *Polymer Chemistry* 6(32), 5827–5831 (2015).
16. Giulbudagian M, Rancan F, Klossek A *et al.* Correlation between the chemical composition of thermoresponsive nanogels and their interaction with the skin barrier. *J. Control. Release* 243, 323–332 (2016).
17. Rancan F, Giulbudagian M, Jurisch J, Blume-Peytavi U, Calderon M, Vogt A. Drug delivery across intact and disrupted skin barrier: identification of cell populations interacting with penetrated thermoresponsive nanogels. *Eur. J. Pharm. Biopharm.* 116, 4–11 (2017).
18. Rancan F, Volkmann H, Giulbudagian M *et al.* Dermal delivery of the high-molecular-weight drug tacrolimus by means of polyglycerol-based nanogels. *Pharmaceutics* 11(8) (2019).
19. Gerecke C, Edlich A, Giulbudagian M *et al.* Biocompatibility and characterization of polyglycerol-based thermoresponsive nanogels designed as novel drug-delivery systems and their intracellular localization in keratinocytes. *Nanotoxicology* 11(2), 267–277 (2017).
20. Edlich A, Gerecke C, Giulbudagian M *et al.* Specific uptake mechanisms of well-tolerated thermoresponsive polyglycerol-based nanogels in antigen-presenting cells of the skin. *Eur. J. Pharm. Biopharm.* 116, 155–163 (2017).
21. Giulbudagian M, Honzke S, Bergueiro J *et al.* Enhanced topical delivery of dexamethasone by beta-cyclodextrin decorated thermoresponsive nanogels. *Nanoscale* 10(1), 469–479 (2017).
22. Sahle FF, Giulbudagian M, Bergueiro J, Lademann J, Calderon M. Dendritic polyglycerol and N-isopropylacrylamide based thermoresponsive nanogels as smart carriers for controlled delivery of drugs through the hair follicle. *Nanoscale* 9(1), 172–182 (2017).
23 . Jung S, Nagel G, Giulbudagian M *et al.* Temperature-enhanced follicular penetration of thermoresponsive nanogels. *Zeitschrift für Physikalische Chemie* 232(5–6), 805–817 (2018).
24. Sonzogni AS, Yealland G, Kar M *et al.* Effect of delivery platforms structure on the epidermal antigen transport for topical vaccination. *Biomacromolecules* 19(12), 4607–4616 (2018).
25 . Clichici S, Filip A. *In vivo* assessment of nanomaterials toxicity. In: *Nanomaterials – Toxicity and Risk Assessment.* Soloneski S, Larramendy ML (Eds). IntechOpen, London, UK (2015).
26. D'Mello SR, Cruz CN, Chen ML, Kapoor M, Lee SL, Tyner KM. The evolving landscape of drug products containing nanomaterials in the United States. *Nat. Nanotechnol.* 12(6), 523–529 (2017).
27. Prow TW, Grice JE, Lin LL *et al.* Nanoparticles and microparticles for skin drug delivery. *Adv. Drug Deliv. Rev.* 63(6), 470–491 (2011).

10.2217/nnm-2019-0345