ABSTRACT

**Background:** Topical Delivery in photodynamic therapy (PDT) is a complicated task with often unsatisfactory results. **Objective:** Developing Indocyanine green (ICG) transfersomal hydrogel to enhance its dark- and photostability, as well as improving its topical delivery as photodynamic sensitizer. **Methods:** Two transfersomal preparations entrapping ICG either in the lipid membrane or the aqueous core were formulated. Both formulations were studied, for some pharmaceutical properties, entrapment efficiency for ICG as well as ICG dark- and photostability. The formula of higher loading capacity for ICG, longer shelf life time (in dark) as well as photostability, was formulated in topical hydrogel and further studied for in vivo permeation study on dorsal mice skin and evaluated clinically for PDT of acne vulgaris, after irradiation with 820 nm using diode laser. **Results:** Entrapping ICG in transfersomes enhanced its dark- and photostability. Histological examination of mice dorsal skin demonstrated that topical PDT with transfersomal ICG provided photodamage at deeper regions of mice skin in comparison to aqueous ICG hydrogel. Clinical evaluation revealed marked improvement in the inflammatory lesion (papules and pustules) counts with more than 80% clearance after 3 sessions till the end of follow up period compared to free ICG gel which showed 45% improvement at the end of the follow up period. **Conclusion:** Formulating of ICG in transfersomal hydrogel significantly increased its
shelf life time and photo-stability. Moreover, it improved its photodynamic properties as a topical sensitizer for treatment of acne vulgaris, compared to aqueous ICG hydrogel.

**KEYWORDS:** Indocyanine green. Transfersomes. Photostability. Photodynamic therapy

**INTRODUCTION**

Acne Vulgaris is the most common skin disease to affect younger humans. Different therapeutic approaches have been introduced into clinical practice. For severe acne vulgaris, isotretinoin is the most effective treatment, with 80–90% reduction of acne lesions. Also, Antibiotics are considered to be the mainstay for treatment of acne. Different investigators have found a reduction of 45–66% of inflammatory lesions after 12 weeks of treatment with standard antibiotic therapy although their success rate varied considerably, due to patient compliance and the increasing antibiotic resistance of P. acnes. The emergence of bacterial resistance is a potential problem associated with prolonged antimicrobial use. The excessive use of tetracycline is also an environmental concern since they remain in nature for a long time before they are eliminated.

Hence, PDT may be an interesting alternative. The renewed interest in antimicrobial PDT originates from two main factors: first, the promising results obtained by PDT in the fields of treatment of skin cancer for which it is now used in routine clinical practice and secondly, the need for new antimicrobial therapies. Previous studies concluded that ALA-PDT may be effective for acne but significant adverse effects associated with treatment, such as erythema and oedema, burning and pain sensation have been reported during illumination.

Indocyanine green (cardio-green, ICG) is FDA approved tricarbocyanine dye, for medical diagnostic studies such as, evaluation of cardiac output and liver function. The important motivation for using ICG as contrast agent for in-vivo imaging is its strong absorption in the near-IR region of the spectrum which leads to strong near-IR fluorescence emission where most biomolecules have low auto-fluorescence which provides relatively free intrinsic background interference, thus, enhancing signal selectivity and increasing imaging depth. Moreover, ICG has a great potential for application in photodynamic therapy (PDT) due to its strong absorption in the therapeutic window region where the penetration depth of light in biological tissue is the highest. However, the in-vitro and in-vivo instability of ICG (aqueous-instability, photo-degradation and thermal-degradation) and its short circulation half-life (plasma t1/2 = 2–4 min) are major hurdles in its
effective use. Thus, a topical ICG dosage form with satisfactory drug stability and high penetration depth into and through intact skin would be of great interest.

Recent approaches in modulating vesicular systems to deliver drugs into deeper tissues have resulted in the design of a novel type of liposomes called transfersomes (deformable liposomes). They are the first generation of elastic vesicles introduced by Cevc.\cite{16, 17} and were reported to penetrate intact skin carrying therapeutic concentrations of drugs when applied under non-occluded conditions.\cite{11, 12} These liquid-state vesicles have a highly deformable membrane\cite{16-19} which permits their easy penetration through skin pores much smaller than the vesicles’ size owing to the added single chain surfactant which acts as an edge activator that destabilizes the transfersomal membrane and increases deformability by lowering interfacial tension.\cite{20}

The main focus of the present study is to develop transfersomes as potential carriers for ICG to enhance its stability and skin penetration depth.

Topical delivery of both free (aqueous) and transfersomal (trans) ICG for PDT was performed on mice and the efficiency of skin permeation and photodynamic activity of ICG in both forms were assessed by histopathological examination. Furthermore, clinical evaluation of the effectiveness of topical PDT mediated by ICG was performed on volunteer patients complaining of mild to moderate degree of acne vulgaris.

MATERIALS AND METHODS

Materials

Indocyanine Green (ICG), Cholesterol (CHOL) and HEPES buffer were purchased from Sigma Aldrich (products of USA). 3-sn-phosphatidylcholine from soy beans (Soy PC) and Deoxycholic acid sodium salt (SDC, ≥98%) were supplied from Fluka Biochemika (products of Italy). Carboxy methylcellulose sodium salt (CMC) were purchased from El Nasr Pharmaceutical Chemicals Co. (Adwic, Egypt).

Methods

Indocyanine green loaded transfersomes preparation

Transfersomes, composed of Soy PC and 10% SDC (w/w of Soy PC), were prepared by the traditional thin lipid film hydration technique (16) according to compositions listed in Table 1.
Table 1: The composition, the entrapment efficiency and the mean particle size of transfersomal formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>PC:SDC:ICG</th>
<th>Entrapment Efficiency ** (% ± SD)</th>
<th>Particle Size before filtration * (nm ± SD)</th>
<th>Particle Size after filtration * (nm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>100:10:5</td>
<td>15.1±0.54</td>
<td>134.07±11.82</td>
<td>138.65±4.25</td>
</tr>
<tr>
<td>T2</td>
<td>100:10:5</td>
<td>49.17±4.07</td>
<td>131.89±12.85</td>
<td>139.51±3.92</td>
</tr>
</tbody>
</table>

Each value given in the table was calculated from n=3 parallels, and are given by the Mean ± SD. Statistical analysis: Paired-Samples t-test using SPSS software, version 16.0, (**P < 0.01,*P < 0.05)

Briefly, appropriate amounts of lipids were dissolved in diethyl ether in a round-bottomed flask. In rotary evaporator (Heidolph VV 2000, Germany), the organic solvent was completely evaporated under vacuum and the formed lipid film was subsequently hydrated by 50 ml freshly prepared HEPES buffer (pH= 7.8) at 40°C. The amphiphilic ICG was added to lipid mixture before evaporation in case of formulation T2 where ICG resides within the lipid membrane or in the aqueous hydration medium in formulation, T1, where ICG is incorporated in the aqueous core of transferosomal vesicles. The resulting vesicles were left to swell for 2 h at room temperature and then stored at 4°C. For preparation of free-ICG and ICG-trans hydrogels Methylparaben and propylparaben were dissolved in appropriate volume of free-ICG aqueous solution or ICG-trans, then carboxy methylcellulose sodium salt (CMC 5%), was added while stirring, and stored overnight at 4°C. Gels with 0.1% ICG concentrations were prepared for in-vivo studies while gel with 6.5*10^{-4} % ± 0.5 (6.5 µg/gm) ICG, were prepared for stability studies.

**Transfersomes characterization**

**Entrapment efficiency**

Prepared vesicular formulations were separated from the unentrapped dye by centrifugation at 10,000 rpm in for 10 min at 10 °C for 10 min by means of a temperature controlled centrifuge (Centrikon T-42K, Kontron Instruments, Watford, Herts, United Kingdom). The transfersomal residue was washed twice with 10 ml HEPES buffer and recentrifuged for further 10 minutes. Aliquots (100 µl) from washed and unwashed transfersomes were dissolved in absolute ethanol and the concentration of each sample was measured by spectrophotometry using UV-VIS double beam spectrophotometer (Ray Leigh UV-2601, USA), at maximum absorption wavelength (788 nm) using the ICG molar extinction...
The percentage Entrapment Efficiency (%EE) of ICG was calculated as follows:

\[
\% E.E = \frac{\text{amount of ICG in washed sample (mg)}}{\text{amount of ICG in unwashed sample (mg)}} \times 100
\]

**Particle Size Measurements and Deformability Test**

The deformability of the prepared transfersomes was determined by measuring the mean size of the freshly prepared transfersomes before and after filtration through a microporous filter with pore size of 100 nm (Whatman nucleopore, Avanti) using a stainless steel filter holder connected to 0.5 MPa pressure source. The size of the prepared transfersomes were determined by optical microscope inverted microscope (Olympus CKX41) supplied by a 650A Power Shot IS Canon digital camera and the recorded images were analyzed for particle size measurement by means of Micrometrics SE/CMOS version 2.6, Accu-Scope, Inc. software.

**In-vitro release study**

ICG release from transfersomes was determined by the dispersion method. Aliquots (2 ml) of each formulation were suspended in HEPES buffer and incubated in a temperature controlled water bath maintained at 37°C with continuous stirring. At predetermined time intervals (15, 30, 45, 60, 90, 105 and 120 min), the incubated suspensions were centrifuged at 10,000 rpm at 15°C for 10 minutes, the maximum absorption of ICG in the supernatant was measured by spectrophotometry at 782 nm and the amount of ICG released in the supernatant was calculated from previously prepared a standard calibration curve of ICG in HEPES buffer at maximum absorption \((\lambda_{\text{max}}=782 \text{ nm})\).

**In-vitro stability study**

Dark- and Photo-stability studies were performed for ICG in aqueous solution (HEPES buffer, PH 7.8) and ICG encapsulated in transfersomes (T1 & T2). The stability of free ICG and transfersomal ICG in the final hydrogel also studied. Samples from each preparation, with initial ICG concentrations 6.5 µg/ml ± 0.5, were kept in dark at room temperature and others were exposed to light at 820 nm emitted from 500-mW diode laser emitting light (Gallium midi 33 diode cluster system, Sim-med Ltd, Billingshurst, West Sussex, United Kingdom, at fluence rate 320 mW/cm², while stirring. At predetermined time points, aliquots (100 µl) from each sample were withdrawn, and the concentration of ICG was
measured by spectrophotometry and expressed as percentage ICG remained at each time point.

**In-vivo evaluation of topical transfersomal ICG delivery into mice skin**

To evaluate transferosomal hydrogel delivery of ICG into skin layers, topical photodynamic therapy on animal skin was carried out.

Twenty four male albino mice (average weight 27 g ± 3) were housed under standard laboratory conditions, at room temperature with a regular light/dark cycle and free access to food and water. All experiments were performed following the “principles of laboratory animal care” (NIH publication nos. 85–23, revised 1985), as well as specific institutional laws on “protection of animals” under the supervision of authorized investigators. The animals were divided into 3 groups, each, of 8 mice.

Groups 1 and 2 included animals on which the gel formulations containing 0.1% free and transfersomal ICG (T2), respectively, were topically applied on shaved dorsal skin for 1 h on 1 cm$^2$ area. The skin was then washed with saline and then 3 animals from each group were irradiated for 2 min by 820 nm diode laser at a fluence rate 320 mW/cm$^2$ (energy delivered= 38 J/cm$^2$) and the other 3 animals were left unirradiated. Group 3 included animals used as control and received neither ICG nor laser.

Animals were sacrificed 24 h post experiments and skin sections were obtained for histopathological examination. The collected skin sections were fixed in Dietrich’s solution, dehydrated, and included in paraffin. Sections of 7 μm thickness were finally stained with hematoxylin–eosin, and then examined by means of inverted microscope (Olympus CKX41) supplied by a 650A Power Shot IS Canon digital camera.

**Evaluation of topical ICG on patients complaining of acne vulgaris**

A randomized, controlled, split-face, single-blinded clinical trial was performed on twenty female patients with an age ranged between 18-25y, skin type (III-IV), complaining of mild to moderate degree of acne vulgaris according to Burtons classification. Their inflammatory lesions ( papules and pustules ) were recalcitrant to standard treatment and were poor candidates for systemic retinoid treatment. Patients were randomly divided equally into two groups, group (A) and group (B). Both groups received topical ICG gel for 15 minutes under dark occlusion but not adhesive dressing followed by diode laser (820 nm)
irradiation for 8 min with a total fluence 153.6 J/cm² to one side of the face while the contralateral side of the face remained untreated, serving as an internal control. A randomized code determined which side of each patient’s face was to be treated.

For group (A), patients received 0.1% ICG-trans topical gel (T2 gel) while group (B) patients received 0.1% free-ICG topical gel. Patients received a total of three treatment sessions spaced at approximately 1-week interval. All patients were followed up for 8 weeks after last treatment session. Strict light precautions were required for the patients for at least 48 h after each treatment session.

Each patient’s acne was visually assessed by a spot count of inflammatory lesions only, as white comedones were not included in this study, at baseline and then every 1 week until the end of the follow up period. In addition, patients and a blinded investigator had to decide whether or not the patient’s acne condition had improved.

Responses were graded: 0= acne worse, 1= no change, 2= mild improvement, 3= moderate improvement, 4= marked improvement and compared the patient’s condition with the baseline photograph.

RESULTS

Figure 1: Absorption spectra of ICG in transfersomes (T1 & T2) and HEPES solution, (empty transfersomes and HEPES buffer were used as blank, respectively).

Transfersomes characterization
The absorption spectra of ICG-trans were scanned and compared with that of free-ICG in HEPES. ICG inclusion in transfersomes shifted the maximum absorption to longer
wavelength (804 nm), compared to the absorption maximum of free-ICG in HEPES (782 nm) (Figure 1).

As seen in table 1, the % EE of ICG loaded in transfersomal membrane (T2), was significantly higher than that of ICG (**P < 0.01) included in the aqueous core (T1). The average particle size of transfersomal formulations was 132.89 nm. There was insignificant difference in the average size of the two formulations (*P < 0.05), with nearly the same size before and after filtration. Moreover, the complete passage of all transfersomal formulations through membranes with pore size smaller than their own size, confirmed the flexibility of the vesicles’ membrane, which is attributed to the surfactant (SDC).[20]

**In-vitro release study**

The release profiles of ICG from transfersomes in (Figure 2) show approximately 70-80% of ICG released within 2 h.

![Figure 2: ICG transfersomes release profiles](image)

**In-vitro stability study**

As presented in (Table 2), ICG in dark underwent significant degradation in aqueous medium at 24°C ($t_{1/2}$= 0.4 d). Incorporating ICG within transfersomal vesicles, either in the lipid membrane (T2) or in the aqueous core (T1) significantly increased the dark stability (**P < 0.01) of ICG ($t_{1/2}$= 24.46 and 15.91 d, respectively). Moreover, formulating free-ICG and ICG-trans (T2) in CMC gel significantly enhanced ICG dark stability (**P < 0.01) compared to their corresponding preparations not formulated in gel; under the same conditions. Moreover, ICG in the transfersosomal gel (T2) preserved its initial concentration for 8 months.
without significant change (**P < 0.01) while free-ICG gel lost about 40% of its initial concentration after the same storage period.

Irradiation of ICG samples by a selective laser beam at 820 nm significantly accelerated ICG degradation (**P < 0.01). However, entrapment of ICG in transfersomal membrane offered efficient protection against photodegradation (T2 \( t_{1/2} = 5.07 \times 10^{-3} \) d) compared to ICG aqueous solution (\( t_{1/2} = 3.10 \times 10^{-3} \) d) (*P < 0.05).

**Table 2: Half-life of ICG in different formula in dark and after irradiation with 820 nm Diode laser.**

<table>
<thead>
<tr>
<th>( t_{1/2} ) (d ± SD)</th>
<th>ICG in HEPES</th>
<th>Formula T1</th>
<th>Formula T2</th>
<th>ICG- trans gel</th>
<th>ICG free Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4±0.09</td>
<td>15.91±2.71</td>
<td>24.46±1.4</td>
<td>&gt; 12 month 100% retained</td>
<td>12.54 months.</td>
<td></td>
</tr>
<tr>
<td>Irradiation by 820 nm</td>
<td>3.10<em>10^{-3} ±6.9</em>10^{-4}</td>
<td>3.93<em>10^{-3} ±1.7</em>10^{-4}</td>
<td>5.07<em>10^{-3} ±6.04</em>10^{-4}</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: not available

**In-vivo evaluation of topical transfersomal ICG delivery into mice skin**

The transfersomal preparation containing ICG in lipid membrane (T2) was used for further in-vivo permeation study and for further clinical evaluation, as it displayed higher entrapment efficiency (49.17%), dark-and photo-stability, compared to T1.

**Figure 3a** shows normal mice skin sections with thin epidermis and dermis which is subdivided into two main areas, an upper papillary region and a deeper reticular region which shows scattered sections of sebaceous glands, normal hair follicles, blood vessels and fat cells.

Histopathological sections of skin treated with either irradiation only or ICG gel only showed no noticeable variation from control skin (figure is not shown).

**Figure 3b** represents skin sections 24 h after PDT using free-ICG gel showing only slight intracellular edema of the papillary dermis (spongiosis) with inflammatory cells infiltrate. The epidermis did not show any sort of discontinuity in response of the inflammatory stimuli. Some skin specimens showed eschar formation with inflammatory cells. On the other hand, irradiated skin sections treated with ICG-trans gel showed normal epidermis, hair follicles and sebaceous glands in the upper part of the dermis while the photodamage was mainly...
confined to the deeper layer of the dermis as all deep hair follicles and blood vessels were damaged (Figure 3c).

**Figure 3: Histopathological sections through the dorsal skin of untreated control mouse skin showing normal aspect for the epidermis, hair follicles, dermis, and normal blood vessels (a). Section taken at 24 h after phototreatment with free-ICG: some skin specimens showed eschar formation with inflammatory cells (b). Sections taken at 24 h after phototreatment with Trans-ICG: the photodamage occurred at lower regionsof the dermis and involved blood vessels and hair follicles photodamage (arrow) (c).**


**Evaluation of topical ICG on patients complaining of acne vulgaris:** In this preliminary study, the number of inflammatory lesions was about the same in regions allocated to ICG trans gel or free ICG PDT. Global severity assessment of acne was graded as moderate in 17 patients and mild in three patients at baseline. Patients treated with ICG trans gel (group A), showed marked improvement with more than 80% clearance of inflammatory lesions after 3 sessions, as shown in **Figure (4 & 5)**. This clearance remains stable for two months follow up after last treatment session. While patients treated with free ICG gel (group B), showed continuous improvement in their treated sides, with 45% clearance in the inflammatory lesions count till the end of follow up period. At the end of follow up period, patients and investigator assessment ranged from moderate to marked improvement for group (A) and from mild to moderate improvement for group (B). In this study, ICG trans gel was significantly more effective than free ICG gel PDT in treating inflammatory lesions, as indicated by a greater median percent reduction in total inflammatory lesion counts at week 8 (80% vs. 45%) as shown in **Figure 6**, and higher patient satisfaction. The treatment sessions were generally tolerated by all the patients with no adverse effects. Only mild itching and transient erythema were experienced by five patients only but resolved within 10-20 minutes.
The untreated half of face of patients of both groups did not show any improvement in acne severity, as shown in Figure 4 (e &f).

Figure 4: Patient complaining moderate acne (± white comedones) right side treated by ICG-trans mediated PDT before (a & c) and after three sessions (b & d). Left side (e&f) served as control without treatment.

Figure 5: Patient complaining mild acne treated by ICG-trans mediated PDT(a, c ) before treatment & (b,d) after three sessions. Black arrows point to acne lesions of the untreated half of the face.
DISCUSSION

Despite its FDA approval as fluorescence biomarker and the promising reports of its photodynamic efficiency,[13-15] ICG has two characteristics which impedes its clinical application: the poor stability of the dye in aqueous solutions, and the short circulation half-life.[8, 15] Thus, formulating ICG in delivery systems with the aim to improve its dark and photo-stability, as well as enhance its topical penetration represent a challenge.

The % EE of ICG loaded in transfersomal membrane, was significantly higher than that of ICG included in the aqueous core. These results are consistent with those obtained by Rengel et al.[23] who examined the encapsulation efficiency of the water soluble, superoxide dismutase prepared by three different vesicle preparation methods and suggested that the thin film hydration technique, used in the present study, constitutes an important obstacle for the entrapment efficiency of water soluble agents.

The average particle size of transfersomal formulations was 132.89 nm, suggesting promising skin penetration as reported by Kang et al. [24] who considered particle size of 130 to 140 nm is ideal for skin delivery.

The release profiles of ICG from transfersomes show that ICG transfersomes exhibited high rate of release from the vesicles. About 70-80% of ICG was released within 2 h which is consistent with all previous studies which reported membrane destabilizing effect of the embedded surfactant (SDC), which lowers the interfacial tension, thus, increases membrane deformability and so dye leakage.[20] The instability of ICG in aqueous solutions was

Figure 6: Differences of reduction of total number of treated acne lesions among two groups.
previously reported, due to physicochemical transformations, such as aggregation, and irreversible degradation, which depend on several factors, solvent nature, concentration, temperature and light exposure.\textsuperscript{15, 25} Saxena et al.\textsuperscript{15} reported that in aqueous solution, at low concentrations (5 mM) of ICG, monomers are prominent, while at higher concentrations (100 mM), ICG oligomers are prominent. Hence, in this work, to study ICG instability due to degradation process only, ICG concentration was maintained at the monomer concentration at 8.4 µM (6.5 µg/ml ± 0.5). The absence of ICG oligomers was confirmed by the absence of its absorption peak at 690 nm\textsuperscript{8}, as shown in Figure 1.

In dark, ICG underwent significant degradation in aqueous medium at 24°C (\(t_{1/2}= 0.4\) d). Holzer\textsuperscript{25} and Saxena\textsuperscript{15} concluded that the dark instability of ICG is due to the irreversible degradation caused by saturation of the double bonds in the conjugated chain of ICG structure, leading to the formation of leucoforms which depends on the presence of solvent radicals and ions, which in case of aqueous solutions, is provided by water. Leucoforms may further degrade to form fragments with intact aromatic end groups.

Incorporating ICG within transfersomal vesicles, either in the lipid membrane (T2) or in the aqueous core (T1) seemed to enhance significantly the dark stability of ICG (\(t_{1/2}= 24.46\) d and 15.91 d, respectively), compared to free-ICG in HEPES buffer (\(t_{1/2}= 0.4\) d). The increased protection of ICG by transfersomes might be explained according to a previous study conducted by Kirchherr\textsuperscript{26}, who concluded that all micellar systems offered a higher stability for ICG than free aqueous ICG via the physical barrier provided by the vesicles’ lipid membranes which isolates the dye from the aqueous environment.

Furthermore, in this study the average half-life of 20.18 ± 2 days obtained by transfersomal ICG stored in dark was superior to that obtained previously by Saxena et al.\textsuperscript{15} for ICG-PLGA nanoparticles aqueous suspension (72.2 h) stored at the same conditions.\textsuperscript{27} Other studies, conducted by Kirchherr\textsuperscript{26}, showed effective ICG stabilization by encapsulation in Solutol HS 15 and Cremophor RH 40 micellar systems with preservation of the initial absorbance of ICG after 4 weeks of storage in dark at 25°C.

Irradiation of ICG samples by a selective laser beam at 820 nm significantly accelerated ICG degradation (**\(P < 0.01\)), compared to their corresponding samples kept in dark. The photoexcited ICG molecules have a tendency to form free radicals and to react with intrinsically present solvent radicals and ions to degrade to leucoforms.\textsuperscript{15, 25}
However, entrapment of ICG in transfersomal membrane offered efficient protection against photodegradation \( (T_2 t_{1/2}=5.07*10^{-3} \text{d}) \) compared to ICG aqueous solution \( (t_{1/2}=3.10*10^{-3} \text{d}) \) \( (*P < 0.05) \). The enhancement in photostability might be due to attenuation of the light reaching the entrapped dye by scattering the incident photons over opaque transfersomes vesicles. This, in addition to the physical protection of the membrane against the aqueous degradation in the dispersion medium leads to delayed degradation, compared to ICG enclosed in the aqueous core or free in aqueous medium.

The transfersomal preparation containing ICG in lipid membrane \( (T2) \) was used for further in-vivo permeation study and for further clinical evaluation. Irradiated skin sections treated with ICG-trans gel showed normal epidermis, hair follicles and sebaceous glands in the upper part of the dermis while the photodamage was mainly confined to the deeper layer of the dermis as all deep hair follicles and blood vessels were damaged compared to superficial damage of sections treated with free-ICG, reflecting enhanced and deeper penetration of transfersomal ICG through skin barriers due to their high deformability and flexibility.\(^{[16]}\)

Valery \(^{[23]}\) hypothesized that ICG mediated PDT using low light intensity protocol \((1.5-30 \text{ J/cm}^2)\) causes inhibition of acne lesions by suppression of *Propionibacterium acnes* (*p. acne*) colonies without cellular damage or modifications to sebaceous glands or canals as this irradiation dose should be sufficient to kill bacteria, but not enough to damage keratinocytes and other tissue components including sebocytes.

Hence in the present study, the conditions used for evaluating ICG mediated PDT on acne patients were upgraded to the medium-intensity protocol of acne treatment \((150-190 \text{ mW/cm}^2)\) which was also introduced by Tuchin.\(^{[5]}\)

Our preliminary results show that free ICG-PDT might be as effective as oral antibiotics, while transfersomal ICG-PDT pilot results might be nearer to those of isotretinoin excluding its major side effects such as dryness or irritation of the skin and mucous membranes, pain or stiffness of bones, joints and muscles, increased blood lipid levels and, more seldom, low counts of white and red blood cells.\(^{[3]}\)

The photodynamic properties of ICG have been investigated *in vitro* \(^{[9, 10, 3]}\) and recently topical application of ICG and diode laser irradiation for acne treatment was described as a new approach for selective photothermolysis of the sebaceous glands.\(^{[28,29]}\) Hence, we assumed that local photodynamic and /or photothermal reactions induced by ICG, could kill
the pathogenic bacteria as *P. Acnes* and modify sebaceous gland SG apparatus functioning, provided that ICG is deeply delivered and accumulated into tissue and interacts with NIR light. Such approach was fulfilled in this work by loading ICG in transfersomes, and was supported by the clinical results which showed more reduction % in the lesion count with higher grade of patient satisfaction, till the end of follow up period with lower number of sessions, (three sessions) compared with the eight sessions obtained in previous studies.\[5,30\]

**CONCLUSIONS**

Loading ICG in transfersomes significantly improved ICG dark stability (up to 1 year at 4°C), as well as its photo-stability and hence its photodynamic efficiency as a topical photosensitizer for treatment of some skin pathologies.

**REFERENCES**


