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ORIGINAL ARTICLE

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In vivo detection of changes in cutaneous carotenoids after chemotherapy using shifted excitation resonance Raman difference and fluorescence spectroscopy

Sora Jung[1] | Maxim E. Darvin[1] | Johannes Schleusener[1] | Gisela Thiede[1] | Juergen Lademann[1] | Marcel Braune[2] | Martin Maiwald[2] | Bernd Sumpf[2] | Günther Tränkle[2,3] | Dunja Kutzer[4] | Felia Elban[1] | Harald Fuss[4]

[1]Department of Dermatology, Venerology and Allergology, Center of Experimental and Applied Cutaneous Physiology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2]Ferdinand-Braun-Institut, Leibniz-Institut für Höchstfrequenztechnik, Berlin, Germany
[3]Fakultät IV, Technische Universität Berlin, Berlin, Germany
[4]Department Hematology and Oncology, Helios Klinikum Bad Saarow, Bad Saarow, Germany

Correspondence
Sora Jung, Department of Dermatology, Venerology and Allergology, Center of Experimental and Applied Cutaneous Physiology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany.
Email: sora_jung@web.de

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Abstract

Background: Various cutaneous toxicities under chemotherapy indicate a local effect of chemotherapy by secretion after systemic application. Here, changes in the fluorescence and Raman spectral properties of the stratum corneum subsequent to intravenous chemotherapy were assessed.

Methods: Twenty healthy subjects and 20 cancer patients undergoing chemotherapy were included. Measurement time points in cancer patients were before the first cycle of chemotherapy (T_{base}) and immediately after intravenous application of the chemotherapy (T_1). Healthy subjects were measured once without any further intervention. Measurements were conducted using an individually manufactured system consisting of a handheld probe and a wavelength-tunable diode laser-based 488 nm SHG light source. Hereby, changes in both skin fluorescence and shifted excitation resonance Raman difference spectroscopy (SERRDS) carotenoid signals were assessed.

Results: Healthy subjects showed significantly ($P < .001$) higher mean concentrations of carotenoids compared to cancer subjects at T_{base}. An increase in fluorescence intensity was detected in almost all patients after chemotherapy, especially after doxorubicin infusion. Furthermore, a decrease in the carotenoid concentration in the skin after chemotherapy was found.

Conclusion: The SERRDS based noninvasive detection can be used as an indirect quantitative assessment of fluorescent chemotherapeutics. The lower carotenoid SERRDS intensities at T_{base} might be due to cancerous diseases and co-medication.

KEYWORDS
carotenoids, chemotherapy, fluorescence, Raman difference spectroscopy, shifted excitation resonance

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1 | INTRODUCTION

Cutaneous toxicities count to the most frequent side effects during chemotherapy.[1,2] Previous studies showed that intravenously applied chemotherapeutics can be found within the sweat being secreted to the skin surface. The chemotherapeutics subsequently spread on the skin surface as if topically applied and re-penetrate into the upper skin layers.[3] Here, they can lead to radical formation and inflammatory or toxic skin effects, including development of palmar-plantar erythrodysaesthesia, also known as a hand-foot syndrome.[4-10]

The skin of healthy volunteers, especially the stratum corneum layer, usually contains a high concentration of antioxidants. Among them are carotenoids, vitamins, and enzymes, which form an antioxidant network and serve as a part of the body's protective system against free radicals. Recent studies show that carotenoids serve as marker substances of the entire antioxidant status of the epidermis in vivo[11,12] and the kinetics of their degradation in the skin show the intensity of influencing stress factors.[13]

The kinetics of inverse penetration of doxorubicin on the skin surface were described previously.[3] It was found that 30 minutes to 1 hour after systemic administration after chemotherapeutic infusion, fluorescence signals of doxorubicin were detectable on the skin surface.[3] This leads to the conclusion that doxorubicin, like carotenoids and vitamin E, too, is secreted to the skin surface with the sweat, spreads there, and then penetrates into the stratum corneum like topically applied.[14] This result also explains why the dermal side effects associated with systemic administration of doxorubicin occur mainly in the palms of the hands and the soles of the feet. The highest sweat gland density is present at these skin sites[15] so that the proportion of escaping doxorubicin is highest here as well. The horny layer is ten to twenty times thicker on the palms and soles of the feet than on the other areas of the skin, providing an ideal reservoir for the absorption of sweat-derived substances, such as chemotherapeutics in human skin. Depending on the designated chemotherapy schedule and dose, multiple cycles of chemotherapy can cause an accumulation of chemotherapeutic substances[16] resolving in toxic local effects on the skin. However, the specific quantities and dynamics for different chemotherapeutics are not fully understood.[17]

Many chemotherapeutics are Raman-active substances, but their direct detection on the skin is hardly possible due to their low concentration and superposition with the skin Raman spectrum.[18,19] In the case of doxorubicin, absorption bands in the range of 440-520 nm[20] and fluorescence in the range of 520-630 nm[21] are known. This means that the excitation around 488 nm resonantly excites not only the carotenoids beta-carotene and lycopene[22] but also a doxorubicin fluorescence signal[23,24] in the skin. Thus, on the one hand, the doxorubicin fluorescent signal acts as a background signal for the Raman signal. On the other hand, this fluorescent signal makes it possible to detect doxorubicin very sensitively in human skin under in vivo conditions.

Noninvasive reflection spectroscopy was used in a previous study to investigate the decrease in cutaneous carotenoids as a result of increased skin radical formation following intravenous administration of chemotherapeutic agents to the patient's palms. The results clearly showed the significant decrease in cutaneous carotenoids in all intravenously administered chemotherapeutic agents.[25] This clear decrease is detectable using even less sensitive techniques such as reflection spectroscopy.[22] Therefore, a novel diagnostic system would be not only of great importance for the direct detection of doxorubicin on the skin but also for indirect detection of a whole range of other chemotherapeutic agents by measuring their influence on cutaneous carotenoids.

In order to be able to quantitatively determine small changes in the Raman signal intensity of cutaneous carotenoids in vivo, a fluorescence background subtraction procedure should be performed. This can be done by taking advantage of the fluorescence photo-bleaching effect by prolonged exposure of the skin with the reference light.[26] However, this method is time-consuming and did not provide complete subtraction of the fluorescence background.[27] A further method is shifted excitation resonance Raman difference spectroscopy (SERRDS), which provides changes of the excitation wavelength by about 0.4 nm, so that the Raman signals shift along with the excitation wavelength, while the fluorescence bands remain almost constant. By subtracting the two recorded spectra from each other, the fluorescence background is effectively subtracted, and the carotenoid concentration can then be determined by integrating the corresponding Raman bands. The SERRDS device optimized for in vivo measurements of carotenoids in human skin was recently developed by the Ferdinand-Braun-Institute[28] with support of the Einstein Foundation.

Here, changes in the fluorescence and SERRDS signal intensities before and after intravenous chemotherapy were assessed in vivo in cancer patients.

The assessments within this study aimed at determining changes in the carotenoid concentration of the skin as well as detecting different chemotherapeutics by fluorescence changes after intravenous application.

2 | MATERIALS AND METHODS

2.1 | Measurement system

A miniaturized measurement system based on SERRDS[28] was used for the assessment of changes in cutaneous carotenoids and fluorescence signals on the skin surface in vivo. The system uses a measuring spot diameter of 3 mm and a diode laser-based 488 nm SHG light source providing two excitation wavelengths λ_1 = 487.2 nm and λ_2 = 487.6 nm. Here, the fluorescence background can be separated from the Raman peaks. The system was calibrated to a detection limit of 0.03 nmol g^{-1} beta-carotene per gram of skin and was described in detail previously.[28] The carotenoids' signal was recognizable at approx. 1525 cm^{-1}.[22]

2.2 | Study design

A total of 20 healthy subjects and 20 cancer subjects aged from 43 to 77 were enrolled in the study, with each subject group

including 10 male and 10 female subjects of skin type I-III. Cancer subjects suffered from pancreatic carcinoma (25%), mamma carcinoma (15%), non-Hodgkin's lymphoma (10%), liposarcoma (10%), rhabdomyosarcoma (5%), prostate cancer (5%), multiple myeloma (5%), hypopharyngeal carcinoma (5%), and adenocarcinoma of the ovary (5%) and of the cecum (5%) and metastatic adenocarcinoma of unknown primary (5%). The chemotherapies included (pegylated liposomal-) doxorubicin, epirubicin, dacarbacin, vincristin, cycloposphamid, Ifosphamid, topotecan, irinotecan, 5-fluorouracil, (nab-)paclitaxel, docetaxel, carboplatin, and gemcitabine and were applied in an ambulant setting. The patient-related applied chemotherapeutics are summarized in Table 1. All subjects did not receive any chemotherapy within the last 4 weeks prior to the Tbase measurements. The main exclusion criteria were any type of cutaneous toxicity, eczema, or other relevant skin disease within the measurement area.

Measurements in cancer subjects were taken once before systemic intravenous administration (T_{base}) of the chemotherapeutic agent and a second time immediately after systemic administration of the chemotherapy (T_1). The measurements were carried out on both palms at each thenar eminence with five measurements per time point and measuring area.

Healthy subjects were measured at one time point without any further intervention and measurement data compared to those of cancer subjects at T_{base}.

Subsequently, the measured Raman spectra were analyzed for measurement quality and the presence of carotenoid-related Raman bands. The corresponding SERRDS intensity was calculated by integrating the Raman signals around the peak at 1525 cm^{-1} at an excitation wavelength of 487.2 and 487.6 nm and was used for the assessment of carotenoid concentration in the skin. The mean of the SERRDS intensities of all measurements from one measurement area was calculated. In addition to the SERRDS results, the fluorescence intensity at identical position (peak at 526.3 nm: excitation wavelength 487.2 nm, Raman shift 1525 cm^{-1}) was calculated.

The applied chemotherapeutics in 18 out of 20 patients could be divided into two major groups according to their main active ingredients, on the one hand, active ingredients from the group of anthracyclines and on the other hand, the active ingredients from the group of alkaloids (Table 1). Two patients received both anthracyclines and alkaloids. This assignment was used in parts of the evaluation.

TABLE 1 Chemotherapeutics applied in the individual patients

Patient number	Chemotherapeutic substance
1	Irinotecan/folinic acid/fluorouracil
2	Paclitaxel
3	Docetaxel
4	Cyclophosphamide/doxorubicin/vincristine
5	Docetaxel
6	Bortezomib/pegylated liposomal doxorubicin
7	Carboplatin/paclitaxel
8	Pegylated liposomal doxorubicin
9	Cyclophosphamide/pegylated liposomal doxorubicin/vincristine
10	Paclitaxel/cetuximab
11	Protein-bound paclitaxel/gemcitabine
12	Protein-bound paclitaxel/gemcitabine
13	Ifosfamide/epirubicin
14	Protein-bound paclitaxel/gemcitabine
15	Protein-bound paclitaxel/gemcitabine
16	Protein-bound paclitaxel/gemcitabine
17	Doxorubicin/dacarbazine
18	Cyclophosphamide/epirubicin
19	Doxorubicin/dacarbazine
20	Doxorubicin/dacarbazine

2.3 | Ethical approval

Prior to initiation of the study, approval by the independent Ethics Committee of the State Office of Health and Social Affairs Berlin (LaGeSo) was obtained. The study was registered at the European Databank on Medical Devices (Eudamed No. CIV 15-03-013265) and was conducted according to the principles of the Declaration of Helsinki (1996) and Good Clinical Practice Guidelines. All subjects provided written informed consent.

2.4 | Statistical analysis

The descriptive and statistical analysis of the obtained data was conducted using IBM SPSS vs 22. Analysis of SERRDS values was subject to Mann-Whitney *U* test, in which *P*-values of less than .05 were considered to indicate statistical significance.

3 | RESULTS

3.1 | Fluorescence analysis

The mean fluorescence signal intensity increased by 1.2 ± 0.3 at T_1 showing an increase in 14 out of the 20 cancer subjects.

As shown in Figure 1, the majority of patients showed an increase in mean fluorescence intensity after the end of chemotherapeutic treatment at T_1, while the fluorescence intensity in some patients remained almost unchanged (patients 6 and 12) and even decreased in four patients (patients 1, 3, 13, and 15). The highest increases in fluorescence signal were seen in patients receiving doxorubicin, a member of the anthracycline group, which is known for generating its own fluorescence, which was expected to increase the fluorescence of the skin within these investigations. Accordingly, an average increase of 1.3 ± 0.1 was assessed for patients receiving doxorubicin (patients 4, 9, 17, 19, and 20). A

minor mean increase in fluorescence by a factor of 1.1 ± 0.1 was observed in patients receiving pegylated liposomal doxorubicin (Caelix®) (patients 6 and 8). One explanation for this may be a minor release of doxorubicin in the liposomally encapsulated form. Patients treated with the doxorubicin-related chemotherapeutic agent epirubicin, which is also known to have autofluorescent properties, also showed a small increase in fluorescence (patient 18). The single, distinct decrease in fluorescence was found in one patient of the anthracycline group after treatment with epirubicin (patient 13), which can only be explained by differences of the individual metabolism and kinetics.

Patients who received chemotherapeutics of the alkaloids group (eg, paclitaxel, docetaxel, nab-pablictaxel, or topotecan) showed a mean increase in the fluorescence of the skin by a factor of 1.3 ± 0.2 despite the lack of fluorescence of the drugs themselves within the measurement time points 1 hour after termination of the infusion of the chemotherapeutic agents. Patients treated with the chemotherapy drug nab-paclitaxel (abraxane, patients 11, 12, 14, 15, and 16) showed hardly any change in the fluorescence signal at the end of treatment (mean change 1.1 ± 0.1).

Conflicting results of skin fluorescence observed in two patients of the alkaloids group (patients 1 and 3) after administration of the chemotherapy with fluorouracil and docetaxel, can only be explained by the individual metabolism and kinetics.

3.2 | SERRDS analysis: Carotenoids

Healthy subjects showed a mean SERRDS carotenoid intensity at 1136.4 a.u., which was significantly higher ($P < .001$) than cancer subjects before chemotherapy at T_{base} with a mean SERRDS carotenoid intensity of 435.6 a.u. (Figure 2).

As shown in Figure 3, the intensity of the SERRDS signals, representing the concentration of carotenoids, decreases in the majority of patients (13 out of 20) after administration of chemotherapy.

Patients receiving anthracyclines showed a mean decrease in SERRDS signal intensity of carotenoids of 32.8 a.u., while a mean decrease of SERRDS signal intensity in patients receiving alkaloids was found at 21.6 a.u. (Figure 4).

Furthermore, it can be stated that a higher decrease in SERRDS intensity can be observed in patients who have already been chemotherapeutically treated at an earlier stage compared to patients receiving chemotherapy for the first time.

The degree of carotenoid degradation, which correlates with the decrease in SERRDS intensity, appears to reflect the individual state of the skin's antioxidant protection system, which may be dependent on the stage of tumor development, number of prior chemotherapeutic treatments, and lifestyle, including an antioxidants containing diet.

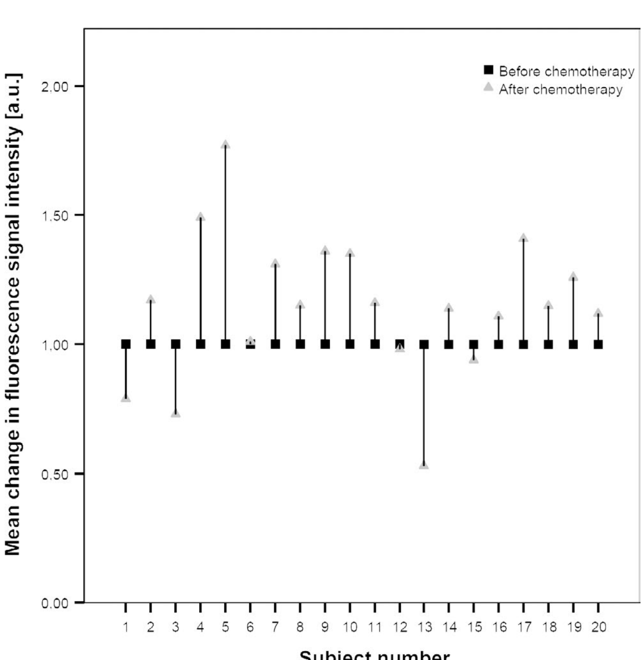

FIGURE 1 Mean changes in normalized fluorescence intensities in each subject at basement measurements (T_{base}, black squares) and after completion of intravenous administration of respective chemotherapeutic agent (T_1, gray triangles). Shown are the calculated mean values of the fluorescence intensities of the left and right thenar eminence

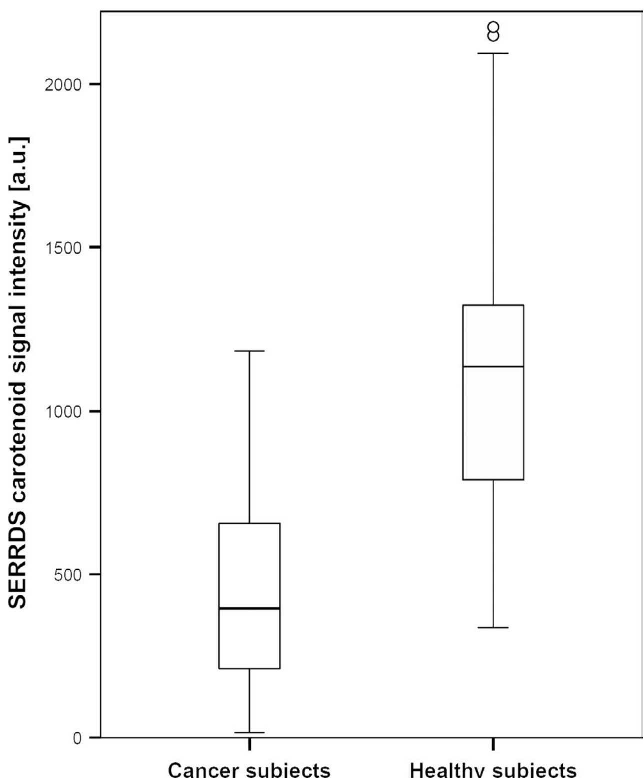

FIGURE 2 Boxplot of SERRDS carotenoid signal intensities in cancer subjects prior to the first cycle of chemotherapy at T_{base} and healthy subjects showing a significant difference. Cancer subjects received no chemotherapy 4 weeks prior to the measurements at T_{base}

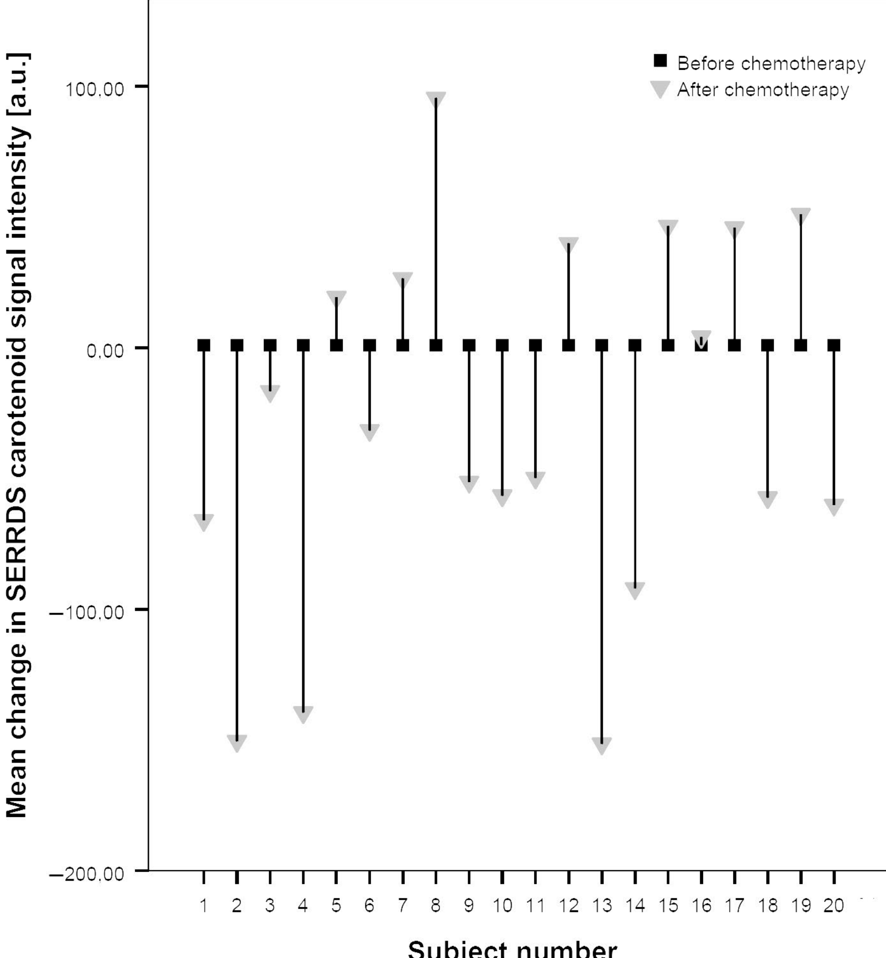

FIGURE 3 Mean changes in SERRDS signal intensities of cutaneous carotenoids at basement measurements (black squares) and after intravenous chemotherapy administration (gray triangles). Shown are the calculated mean values of the SERRDS carotenoid intensities of the left and right thenar eminence

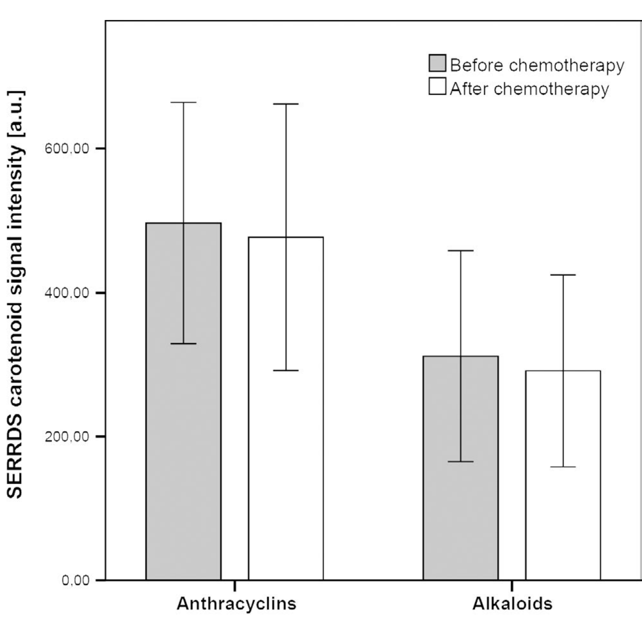

FIGURE 4 Mean SEERDS carotenoid signals in patients receiving chemotherapy with anthrycaclins and alkaloids. Both groups show an overall decrease in SERRDS signal intensities. The error bars represent the 95% confidence interval

4 | DISCUSSION

In summary, the applied SERRDS measurements showed excretion of intravenous chemotherapeutic agents on the skin surface by simultaneously analyzing the carotenoid antioxidants and the fluorescence in the skin. The measurement system could be used for the indication to initiate a prevention strategy of cutaneous toxicities in the form of topical application of antioxidants.[29,30]

The results obtained show that some of the fluorescence-active chemotherapeutic agents, for example, doxorubicin or epirubicin can be detected due to enhanced skin fluorescence as expected.[31]

The direct detection and identification of all fluorescence-free chemotherapeutic agents in the skin using SERRDS was not observed within this study and can be subjected to further investigations.

Nevertheless, indirect measurement of chemotherapeutic agents on the skin surface is possible by SERRDS measurements of epidermal carotenoid antioxidants, which are diminished by interaction with the chemotherapeutic agents. This confirms the assumption that topically applied chemotherapeutic agents that reach the skin surface through systemic administration by sweat or sebum are

able to induce oxidative stress conditions in the skin.[32] The generated radicals react as topically applied with the skin's antioxidants in the stratum corneum, which are subsequently decreased. By reducing the level of antioxidants, the protective function of the skin is weakened and the induction of skin damage, inflammation, or skin toxicity, as shown for the hand-foot syndrome, can increase with each chemotherapeutic cycle.

The distinct variation in carotenoid concentration between subjects is due to interindividual differences in dietary behavior and lifestyle, as known from a number of other studies conducted in this field.[33-38]

Furthermore, it can be stated that cancer subjects already show significantly lower SERRDS carotenoid intensities before chemotherapy treatment compared to healthy subjects. This can be due to the cancerous disease itself and associated emotional burden and stress,[39-41] due to co-medication or even to prior chemotherapeutical treatments at an earlier stage.

The level of carotenoid degradation after chemotherapy that correlates with the decrease in SERRDS intensity appears to reflect the individual state of the skin's antioxidant protection system, which may be dependent on the stage of tumor development, number of prior chemotherapeutic treatments, lifestyle, and antioxidant diet.

Interestingly, patients who received chemotherapy for the first time showed a higher increase in fluorescence intensity. This may be due to the fact that with each chemotherapy cycle, depending on dosage and cycle intervals, a portion of the chemotherapeutic agent remains in the skin, which serves as a reservoir and thus leads to increased baseline fluorescence intensity.

This measurement setting could be of use in future studies investigating, for example, disruptive effects on the skin barrier subsequent to chemotherapy. Changes in the lipid/keratin concentration of the stratum corneum after completion of the systemic administration of the chemotherapeutic agents can serve as an additional indirect parameter of the influence of the chemotherapeutic agents on the barrier function of the stratum corneum. For optimal determination of lipids and keratin whose Raman signals are broadband (around 200 cm^{-1}),[42] other excitation wavelengths should be used for improved evaluation of the lipid/keratin-related SERRDS signals in further studies.

Such a system could be of great importance not only for the investigation of development of dermal side effects of patients under chemotherapy but for all types of applications and skin diseases that can possibly lead to stratum corneum-related barrier damage.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

ORCID

Sora Jung https://orcid.org/0000-0002-9520-2750
Maxim E. Darvin https://orcid.org/0000-0003-1075-1994
Johannes Schleusener https://orcid.org/0000-0003-4088-6523

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