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Evaluation of Toxicological Risks of Nail Coatings Containing Acrylate Monomer HEMA

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Individuals working in nail salons are exposed to 2-hydroxyethyl methacrylate (HEMA). HEMA has been found to have several effects on the skin: skin itching, peeling, redness and allergic contact dermatitis. The purpose of the study was to compare the effects of nail coatings containing HEMA and 2-hydroxypropyl methacrylate (HPMA) on the skin. In this study we explored the irritation properties of HEMA and HPMA containing nail coatings in cell cultures in-vitro and in skin PATCH tests under dermatological control. The cytotoxicity of coatings was tested in BALB/c3T3 and HaCaT cell lines by a neutral red uptake assay. Cytotoxicity was expressed as a concentration-dependent reduction of the uptake of neutral red, compared to the untreated controls. Open patch tests were supervised by a certified dermatologist. Polymerized coating extracts have little effect on Balb/c 3T3 cell viability, while having mild cytotoxic effects on HaCaT keratinocytes. Among two tested samples, extracts of HEMA containing coating exhibited higher cytotoxicity – reduction of keratinocyte viability by 28.29 % in case of undiluted 24 h extract and even by 48.26 % in case of 50 % extract was observed. Coating cytotoxiaty observed on HaCaT showed that the keratinocyte cell line was more sensitive to HEMA than to HPMA containing coating.

Keywords: coatings, acrylate monomers, cytotoxicity, nail coatings, hydroxyethyl methacrylate.

1. INTRODUCTION

Acrylates are one of the most reactive monomers polymerizing by a free-radical mechanism [1]. Acrylates and methacrylates are present in a variety of commonly used products, such as adhesives, coatings [2], photo-sensitive materials [3], bone cement, dental materials as well as artificial nails and UV-cured nail coatings [2]. Acrylic monomers are considered to be potent sensitizers, which can cause occupational allergic contact dermatitis (ACD) affecting nail technicians, dental personnel, printing or coating workers [4, 5].

The most common agent causing dermatitis among nail technicians is 2-hydroxyethyl methacrylate (HEMA) [6 – 8]. The European Commission's Scientific Committee on Consumer Safety (SCCS) has the opinion that HEMA, when used up to 35 % as part of UV curable nail coating system and applied appropriately to the natural nail plate, won't cause a risk of sensitization. Still the growing popularity of UV curable nail coatings, less accurate use in saloons and rising potential use by customers at home increase the risk of sensitization. Previously done studies by dermatologists show, that there is a high risk of contact dermatitis from UV curable nail coatings, among which HEMA is one of the most often used ingredient [9 – 12].

One of the highest risk zones is the problem of an incorrect application of the coating. Beginner nail technicians and hasty masters may apply the uncured gel not only on the nail plate but also on the surrounding skin. Also, the inaccurate removal of the oxygen inhibition layer after UV curing can cause acrylic contact with the skin around the nail plate. All these factors can promote allergic reactions and contact dermatitis to methacrylate [9].

Due to hydrophilicity, uncured HEMA could diffuse through keratin cells more easily than other monomers [13]. It may versus the skin around the nails, such as itching, peeling and redness.

Previous research was done to analyze patch test results of methacrylate containing products from 2004 to 2013. Were summarized results from 114 440 panelists. Only 2 % of them responded with a positive patch test to HEMA, while among tested 87 nail artists, 31 % are positive [10].

Results of an observational and retrospective study from January 2006 till April 2013 evaluated epidemiological and clinical parameters and positive patch test results from methacrylates. Among 2263 patch-tested patients, 80 % of positive HEMA responses were for beauty technicians working with artificial nails, industrial workers, and dentists [11].

Wide retrospective analysis of patch test results with methacrylates including clinical and demographic data analyzed the frequency of contact allergy to (meth) acrylates used in acrylic nail coatings in manicure technicians as well as in consumers. Totally 72 244 female respondents were patch tested in the period from 2011 to 2015. The researchers accomplish that contact allergy to methacrylates was much more common among nail technicians with suspected allergic contact dermatitis to nail coating composition substances (47.1 %) than among consumers

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with no suspected allergic contact dermatitis to nail coating ingredients (2.0 %) [10, 14].

Scientists in 2016 analyzed and showed that patch test results from HEMA containing materials are positive in more than 90% of studies if patients had the previously detected allergy to methacrylates and 64% of patch testing was positive for 2-hydroxypropyl methacrylate (HPMA) [12]. Methacrylate induced toxicity and sensitization have been studied *in vitro* using various cell cultures. In most studies pure monomers at low concentrations had been tested. The cytotoxic effects varied depending on the chosen cell line and testing protocol. HEMA has been reported to negatively affect the viability of lung alveolar cells, osteoblasts, fibroblasts, immune cells [15 – 19]. Mechanisms underlying HEMA cytotoxicity and sensitization have not been fully characterized yet. The majority of previous studies support the hypothesis that HEMA induces the production and accumulation of reactive oxygen species, produces DNA damage, and interferes with cellular signaling pathways [20, 21]. The release of HEMA from dental bonding resins has been studied, but to our knowledge no studies have been performed with HEMA containing nail coatings.

Previously done research from 1999 till 2019 were studied and summarized results from PATCH tests of HEMA and HPMA containing products. Overall, 16 studies have been summarized, which includes tests on almost 100 thousand participants (woman, aged 20 – 65).

Given the importance of this subject, the aim of our study was to identify the substitute to HEMA and compare it with the second most often used acrylate monomer HPMA in nail coating formulations, comparing the extracts of nail coatings containing different monomers, their cytotoxicity and irritancy on the skin.

2. METHODOLOGY

2.1. Materials

The nail coating composition base was prepared at Kinetics Nail Systems Ltd. There was used the traditional base from urethane acrylate oligomers, photoinitiator, and monomer. As monomers were used HEMA (Esstech Ltd.) and HPMA (Esstech Ltd.) to obtain 2 coating formulations CMC-A22 (contains 30 % HEMA) and AMC-H05 (contains 30 % HPMA) for further tests.

2.2. Cytotoxicity testing

BALB/c 3T3 clone A31 murine fibroblast cell line (American Type Culture Collection), and human skin keratinocyte cell line HaCaT (Cell Lines Service) were used in the study. Cells were propagated in DMEM medium (Sigma, D6046, Irvine, UK) supplemented with 1 % penicillin (100 U/mL) – streptomycin (100 µg/mL) and 10 % fetal bovine serum (Sigma) for HaCaT cells or 10 % calf serum (Sigma,) for BALB/c 3T3 cells. Cells were cultivated in a humidified 5 % CO_2 atmosphere at 37 °C.

The cytotoxicity of coatings in BALB/c3T3 and HaCaT cell lines was tested using by neutral red (NR) uptake assay. Nail coating samples were cut in the pieces of approx. 0.5 x 0.5 cm and extracted with cell cultivation media (0.2 g nail coating sample per 1 ml of cultivation media) for 24 h and 72 h at 37 °C. Extracts were then collected, diluted with cultivation media to 12.5 %, 25 %, 50 % (v/v). 0.1 mL of undiluted extract was then added to the corresponding cell culture wells of a 96-well plate and incubated for 24 h in a humidified 5 % CO_2 atmosphere at 37 °C. Cells incubated without extracts samples were used as untreated controls, 100 µg/ml sodium dodecyl sulphate was used as the cytotoxicity control. After incubation cells were washed with phosphate buffered solution (pH 7.4), and neutral red solution (25 µg/mL in cell cultivation media) was added, and cells were incubated for 3 h in a 5 % CO_2 atmosphere at 37 °C. Neutral red was extracted from cells with 50 % ethanol/ 1 % acetic acid solution and absorbance at 540 nm was measured using a Tecan M200 Infinite Pro microplate reader. Cytotoxicity was expressed as a concentration – dependent reduction of the uptake of NR, compared to the untreated controls.

2.3. PATCH test

The assessment of sensitizing and irritating properties of these products was performed on a group of 25 healthy adult volunteers without allergic history. The product AMC-H05 (a base formulation containing HPMA) and product CMC-A22 (a base formulation containing HEMA) in useful concentration was applied to the skin on the forearm in 3 × 3 cm. Reading the response of the skin was performed 15 min, 30 min, 1 h, 24 h and 48 h after the test application. Based on observation of the skin reaction dermatologist assesses the irritating and sensitizing effects of the tested substance.

2.4. Statistical analysis

Three replicates for each nail coating sample were analyzed. Average ± standard deviation (SD) was used to express the experimental values. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used for statistical analysis. A p-value < 0.05 was considered to be statistically significant ($*p < 0.05$; $**p < 0.01$; $***$ – f or $p < 0.001$). GraphPad Prism 8 software was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Research review

Research data from 1999 to 2019 and patch test results have been analyzed (Table 1). Exploring dermatological tests done over the past 20 years, we analyzed the response of nearly 100.000 people reactions to HEMA and found that for sensitive people HEMA causes skin irritation in 20.3 % of individuals, whereas HPMA causes skin irritation in 7.4 % of cases.

3.2. Cytotoxicity of polymerized nail coatings

To evaluate the cytotoxicity of polymerized nail coatings we adapted the testing approach used for biocompatibility testing of biomaterials. As the risk of toxicity and skin irritation due to nail coating use is associated with leakage of potentially toxic compounds, nail-coating samples were extracted in cell cultivation media and media at various concentrations tested in two cell lines.

Table 1. Overview on patch test results from case studies

Number of patients	No. of positive reactions to monomer				Reference
	HEMA	%	HPMA	%	
475	29	6.1	29	6.1	Spencer 2016 [5]
2357	42	1.8	41	1.7	Gatiga-Ortega 2017, Gatiga-Ortega 2018 [7, 8]
72244	254	0.4	218	0.3	Uter 2015 [10]
2263	30	1.3	29	1.3	Ramos 2014 [11]
220	198	90.0	120	54.5	Raposo 2017 [12]
8	6	75.0	0	0.0	Dahlin 2016 [21]
1400	29	2.1	26	1.9	Tucker 1999 [22]
55	17	30.9	17	30.9	Lazarov 2007 [23]
73	16	21.9	0	0.0	Muttardi 2014 [24]
113	37	32.7	0	0.0	Schnuch 2016 [25]
455	44	9.7	0	0.0	Montgomery 2016 [26]
18228	124	0.7	99	0.5	Goncalo 2017, Goncalo 2018 [27, 28]
1306	125	9.6	62	4.7	Rajan 2018 [29]
5920	102	1.7	61	1.0	Rolls 2019 [30]
99197		20.3		7.4	Total/ average

Two extraction times were chosen to investigate whether the toxic effect might be caused by compounds that are released over shorter or longer periods. As previous studies report variable effects of pure monomers, including cytotoxicity and genotoxicity in different cell cultures, e.g. bronchial and oral epithelial cells, pulp and dermal fibroblasts, osteoblasts, immune cells, [15 – 18], we chose to perform cytotoxicity testing in two different cell lines – one, Balb/c 3T3, a widely used cell line in cytotoxicity assessment, the other, HaCaT keratinocyte, to model potential effects on the skin. Results show that extracts have little effect on Balb/c 3T3 cell viability, while having mild cytotoxic effects on HaCaT keratinocytes (Fig. 1).

Among two tested samples, extracts of CMC-A22 exhibited higher cytotoxicity – reduction of keratinocyte viability by 28.29 % in case of undiluted 24 h extract and even by 48.26 % in case of 50 % extract was observed. Undiluted 24 h extract of AMC-H05 reduced keratinocyte viability by 21.24 %, diluted extracts did not have a negative effect. The negative effect of CMC-A22 on cell viability was also observed in the case of 72 h extracts, however, the decrease in keratinocyte viability was less pronounced. The negative effects of methacrylates on HaCaT keratinocytes at specific concentrations can be explained by the mechanisms characterized in other cell lines. It has been shown before that methacrylate generates oxidative stress, impair mitochondrial functions, and leads to apoptosis of dental pulp cells [31]. Dose dependent effects on mitochondrial metabolism and apoptosis have also been observed in immune cells [32].

Our results indicate that HaCaT keratinocytes can be used as a robust yet sensitive *in vitro* models to evaluate the safety of nail coating components. Results show that the keratinocyte cell line was more sensitive to HEMA containing coating than HPMA containing coating. Slightly lower cytotoxicity of 72 h extracts indicate the potential presence of volatile compounds, when might be in higher concentrations in 24 h extracts than after longer extraction times.

From similar extract studies with dental materials, it is known that HEMA and HPMA in micromolar concentrations can be eluted from polymerized materials. Such eluted concentrations are usually lower than IC_{50} values of pure monomers. Because of this, similarity, as in studies of dental resin extracts, viability was not reduced by more than 50 % [33 – 37].

Results indicate that extract of HEMA containing coating is cytotoxic in keratinocyte cell culture, while the HPMA containing coating does not affect cell viability. Until now there is a lack of studies comparing the same test system these two monomers. Yoshii 1997 reported pure HPMA to be slightly more cytotoxic than HEMA in the HeLa S3 cell line, which is opposite of our observation [35].

An interesting finding is the different response of HaCaT and Balb/c 3T3 cells. With response being more pronounced in a keratinocyte cell line, we can conclude that keratinocytes might be a more relevant test system for cytotoxicity screening of nail coating components and their compositions. To our knowledge, the sensitivity of these two cell lines to methacrylates has not been compared before. In study where four different fibroblast cell lines were compared Balb/c 3T3 cells were found to be the most sensitive compared to the other three when pure monomers were tested [37]. Here we show that keratinocytes are even more sensitive.

3.3. Patch tests of nail coatings

The open Patch test study was performed with the involvement of a dermatologist. The panel group included 25 volunteers. Two compositions containing HEMA and HPMA were evaluated. The study approves, that both nail coating compositions: containing HEMA (CMC-A22) and HPMA (AMC-H05) are well tolerated by the skin. Our panelists were persons whose allergy to acrylates hasn't been documented before and after the test were no irritations or allergic reactions. Previous studies also showed that there is a small risk (2 %) of irritations for persons without a tendency to allergic reactions [10, 12]. Studies show that there is a much higher risk of methacrylate sensitization among beauty specialists and nail technicians or persons who had previous allergic reactions to methacrylates [10 – 12, 14, 23]. Tested coatings meet the requirements of legislation and showed positive results in the required compatibility test with the skin (Skin Compatibility Test),

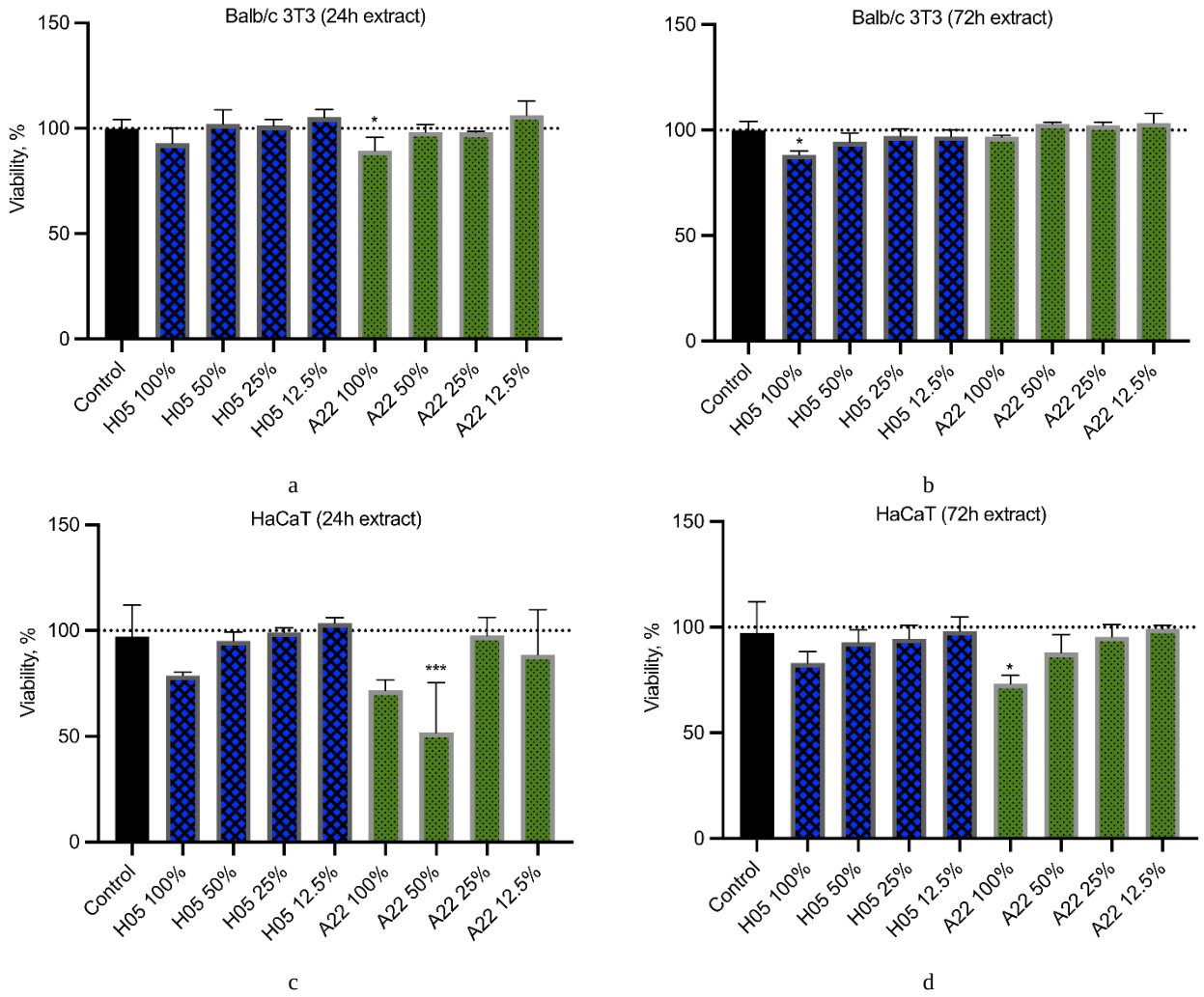

Fig. 1. Changes in the viability: a, b – of Balb/c 3T3; c, d – of HaCaT cells after incubation with nail coatings that were extracted 24 h (a, c) and 72 h (b, d), data show as mean ± SD (n = 3), dashed line indicates control level (100 % viability); ANOVA, * $p < 0.05$, *** $p < 0.001$ compared to control

therefore, it allows the producer to conclude, that both coating compositions can be classified as not irritating.

Also, CIR Expert Panel concluded that HEMA and HPMA are safe as used properly in UV curable nail coating products when skin contact is avoided [9]. Still, there is the risk to have the atopic contact dermatitis or allergic reactions to products containing methacrylates, if there was detected previous sensitization to methacrylates [10, 11, 22]. Our results agreed with the research of Rolls [30] where the higher response to allergic reactions were to HEMA containing material (1.7 %) than for HPMA containing material (1.0 %). Therefore, the producer should inform and educate the beauty technicians regarding the risks associated with the use and proper application of the product.

4. CONCLUSIONS

More often we can face allergic reactions after the manicure procedures with UV curable acrylate nail coatings. The popularity of these coatings and improper usage of nail curing products are the reason for allergic reactions and atopic dermatitis. The industry develops faster than regulatory procedures, therefore high responsibility is on producers. It is the duty of producers to educate the technicians in the beauty industry to apply the nail coatings in properly and to choose the less irritant products.

Evaluation *in vitro* using relevant cell cultures as test systems can serve for safety assessment purposes as well as can help manufacturers and beauty product developers to characterize product formulations. In our study effects of two different nail coating formulations were tested and cytotoxic effects showed correlation with previously done research on Patch tests. Results indicate that extract of HEMA containing coating is cytotoxic in keratinocyte cell culture, while the HPMA containing coating does not affect cell viability, indicating the HEMA's potential at concentrations leaking from polymerized nail coatings to induce intracellular oxidative damages and apoptosis.

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