The use of GARDskin™ for skin sensitization evaluation of cosmetic ingredients and "real-life" mixtures


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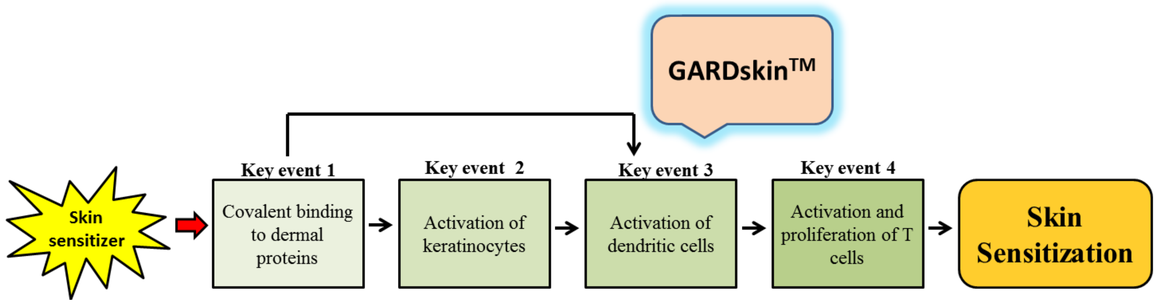
INTRODUCTION

Genomic fingerprints in dendritic cells after chemical exposure is a recent strategy in *in vitro* techniques for skin sensitization hazard. Within this perspective, Genomic Allergen Rapid Detection (GARDskin™), an assay based on a support vector machine (SVM) model, was developed for identifying contact allergens using a myeloid cell line as a surrogate for dendritic cells. Predictive system behind the GARDskin™ consists on the transcriptional quantitative analysis of 200 genes, referred as the GARDskin™ prediction signature. Mechanistically, GARDskin™ is linked to key event 3 "Activation of DCs", as defined by the Adverse Outcome Pathways for skin sensitization published in 2012 by OECD (https://read.oecd-ilibrary.org/environment/the-adverse-outcome-pathway-for-skin-sensitisation-initiated-by-covalent-binding-to-proteins_9789264221444-en#page1) (Figure 1).

Figure 1. Schematic overview of Adverse Outcome Pathways for skin sensitization, published in 2012 by OECD, showing GARDskin™ linked to key event 3.



OBJECTIVES

Given to the wide use in cosmetics field, this study evaluated the applicability of GARDskin™ for evaluation of the skin sensitization potential of hair dye ingredients (n=10) and commercial henna-containing hair colouring mixtures (n=10). Also, the presence of *p*-phenylenediamine (PPD) and lawsone (LAW) in henna products were performed.

METHODOLOGY

After 24 h of exposure with each hair dye ingredient, total RNA extraction of SenzaCells was conducted and gene expression analysis performed using a digital bar-coding platform (NanoString® Technologies) following the method developed by SenzaGen (Lund, Sweden). A test material was classified as a skin sensitizer when the support vector machine (SVM) median output value of the three independent replicates > 0 (Figure 2). Henna-based hair coloring cosmetic products were purchased at the local markets from Goiânia, GO, Brazil. HPs were examined for the presence of PPD and lawsone, a natural pigment/biomarker of henna, by high performance liquid chromatography (HPLC), and then evaluated using GARDskin™.

Figure 2. Schematic overview of workflow of the GARDskin™, developed by SenzaGen.

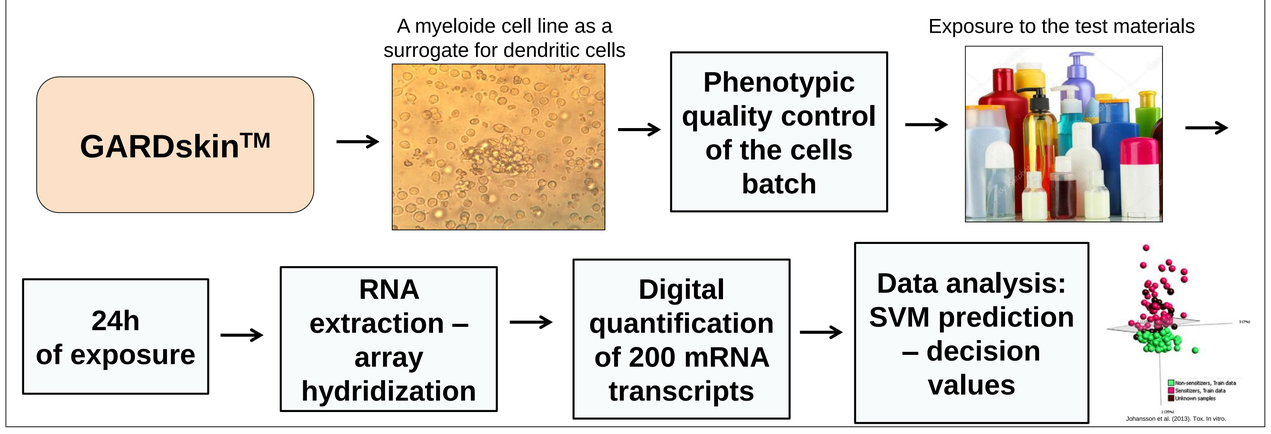
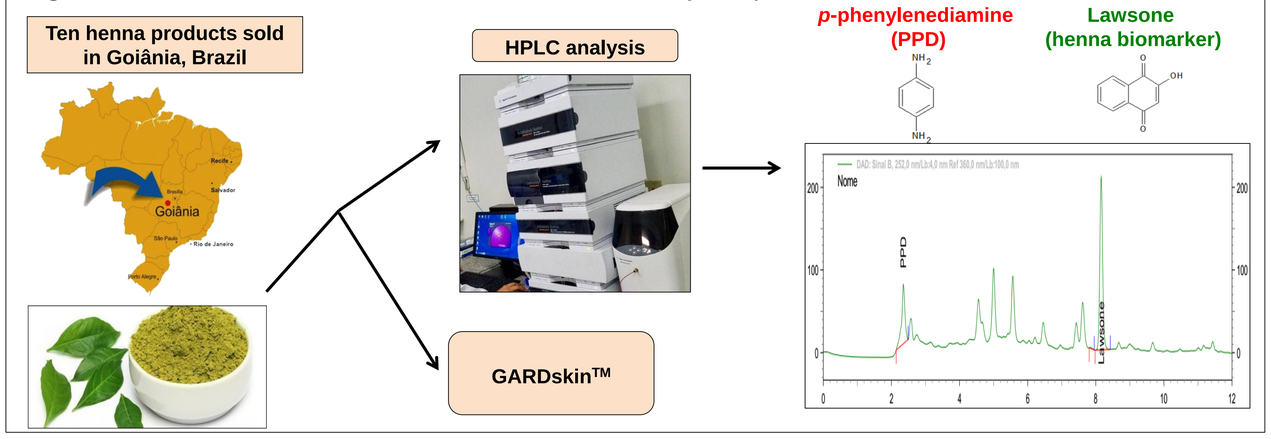


Figure 3. Schematic overview of workflow of the analyzes performed with hennas.



RESULTS

Information declared on the label and lawsone and PDD levels found in ten commercial henna-based hair coloring cosmetics are show in Table 1. Since all products analyzed were declared as henna cosmetics by the manufactures, the presence of LAW, the main active phytochemical of henna, was then expected in all samples. However, HPLC analysis showed no LAW level in the product nº 2, suggesting falsification. Furthermore, the presence of PPD was declared on the products nº 2 and 8 only by the manufactures. However, this substance was detected in all products, suggesting undisclosed adulteration (Table 1).

Table 1. Information declared on the label and lawsone and *p*-phenylenediamine levels found in ten commercial henna-based hair coloring cosmetics.

Product nº	Application	Origin	Ingredients declared on the label: Henna	Other natural materials	PPD	Other synthetic materials	Levels (wt. %): LAW	PPD
1	Hair	Brazil	+				0.518 ± 0.003	1.091 ± 0.028
2	Hair	Brazil	+	+	+	+	ND	2.970 ± 0.046
3	Hair	Brazil	+	+		+	0.188 ± 0.001	0.030 ± 0.001
4	Hair	Brazil	+	+		+	0.320 ± 0.002	0.032 ± 0.006
5	Hair	Brazil	+	+			0.041 ± 0.001	4.321 ± 0.028
6	Hair	Brazil	+	+			0.113 ± 0.001	1.020 ± 0.100
7	Hair	France	+	+		+	0.359 ± 0.001	0.577 ± 0.015
8	Eyebrow	Brazil	+	+	+	+	0.158 ± 0.001	2.541 ± 0.057
9	Eyebrow	Brazil	+	+			0.103 ± 0.001	0.760 ± 0.017
10	Eyebrow	Brazil	+	+			0.032 ± 0.001	3.354 ± 0.163

Abbreviations: +, information declared on the label; ND, not detected; LAW, lawsone; PPD, *p*-phenylenediamine.

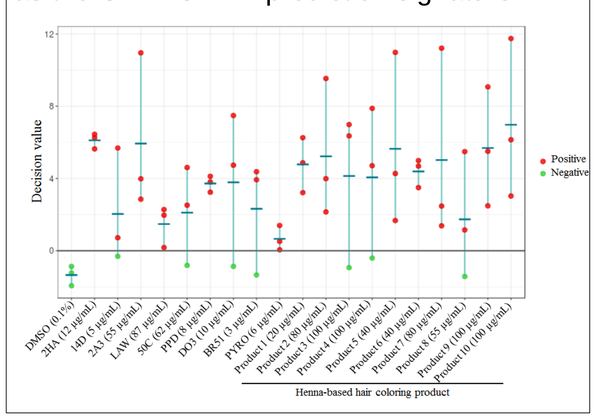
Table 2. Summary of the predictions of the GARDskin™ evaluated for skin sensitization hazard assessment of hair dye ingredients and their concordance in relation to animal and human data.

Test materials	CAS nº	Human classification[1]	Animal classification[2]	GARDskin™
Reference controls				
Dimethyl sulfoxide (0.1%, DMSO)	67-68-5	Negative[a]	Negative[a]	NS
Glycerol (GLY)	56-81-5	Negative[b]	Negative[h]	NS[x]
Sodium dodecyl sulfate (SDS)	151-21-3	Negative[c]	Weak[c]	NS[x,t,u]
1-chloro-2,4-dinitrobenzene (DNCB)	97-00-7	Positive[c]	Extreme[c]	S[x,t]
Eugenol (EUG)	97-53-0	Positive[c]	Weak[c]	S[x]
2-Hydroxyethyl acrylate (2HA)	818-61-1	Positive[d]	Moderate[d]	S
Hair dye ingredients				
1,4-Diaminoanthraquinone (14D)	128-95-0	Positive[e]	Moderate[i]	S
2-Amino-3-hydroxypyridine (2A3)	16867-03-1	Positive[e]	Negative[j]	S
Lawsone (LAW)	83-72-7	NA	Equivocal[k]	S
5-Amino-*o*-cresol (5OC)	2835-95-2	Positive[e]	Moderate[l]	S
Hydroquinone (HQ)	123-31-9	Positive[f]	Strong[m]	S[t,u]
p-Phenylenediamine (PPD)	106-50-3	Positive[g]	Extreme[n]	S
Resorcinol (RSC)	108-46-3	Positive[e]	Strong[o]	S[z]
Disperse orange 3 (DO3)	730-40-5	Positive[g]	Very weak[p]	S
Basic red 51 (BR51)	77061-58-6	NA	Negative[q]	S
Pyrogallol (PYRO)	87-66-1	Positive[f]	Negative[r]	S
Concordance *vs.* Human data			78.5%	100%
Concordance *vs.* Animal data				73.3%

Abbreviations: NS, non-sensitizer; S, sensitizer; NA – data not available by literature; CAS No – Chemical Abstracts Service number. [1]Classification based on the human repeated insult patch test (HRIPT). [2]Classification based on murine local lymph node assay (LLNA) and/or guinea pig maximization test (GPMT)/Buehler test (BT). References: [a]Hartwig and MAK (2017); [b]CIR (2014); [c]OECD (2010); [d]Haneke et al. (2001); [e]Sosted et al. (2013); [f]Uter et al. (2014); [g]Goon et al. (2003); [h]Ryan et al. (2000); [i]SCCS (2010a); [j]SCCS (2008); [k]SCCNFP (2004); [l]SCCP (2006); [m]Kimber et al. (1998); [n]SCCS (2012); [o]SCCS (2010b); [p]Ahuja et al. (2010); [q]CCS (2011); [r]SCC (2000); [s]Johansson et al. (2011); [t]Johansson et al. (2014); [u]Forreryd et al. (2016).

GARDskin™ results are shown in Table 2 and Figure 4. Regarding hair dyes data, GARDskin™ showed a concordance of 73.3% in comparison to animal classification (Table 1, Figure 4). However, this value was 100% when compared to human repeated insult patch test, showing that GARDskin™ prediction correlates with the human classification, in addition to being superior to the animal testing (concordance = 78.5% *vs.* human data) (Table 1, Figure 4).

Figure 4. Effects of hair dye ingredients and henna-based products of transcriptional levels of 200 genomic biomarkers, referred as the GARDskin™ prediction signature.



Moreover, all henna products tested were classified as skin sensitizers, demonstrating that they may not be considered as a safer alternative to synthetic ingredients-based hair dyes, although they are plant-based cosmetics. The hypothesis for these findings seems to be due to the adulteration of the commercial products tested with the synthetic extreme skin sensitizer *para*-phenylenediamine (PPD), as showed by our previous chromatographic analysis.

CONCLUSIONS

Taken together, this study corroborates that GARDskin™ is a promising *in vitro* model to evaluate skin sensitization hazard of cosmetic ingredients. Furthermore, this technology showed suitable to "real-life" mixtures as those one found in the commercial botanical products.

In addition, our findings highlight toxicological consequences of the undisclosed use of PPD in henna-based natural products as well as the risks associated, which may involve sensitization of susceptible consumers and allergic contact dermatitis in previously sensitized individuals. Moreover, our hazard assessment showed that immune responses to these "real-life" mixtures are complex in view of the potentiation effects between henna, synthetic ingredients added and their reaction derivatives formed during the hair dye process.

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