SKIN METABOLISM REVIEW

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ABSTRACT

According to the literature, the metabolism of other organs than the liver such the skin, seems much less studied. Indeed, skin represents the major protective barrier of the body to the environment and chemicals exposure but is not really yet considered as an organ involved in xenobiotic metabolism. It appears to be a tissue of weak catalytic activity generating less diverse metabolites and less funny reaction mechanisms. However, this assertion could be due to the lack of specific tools to study skin metabolism such as particular sample preparation protocols or analytical methods which are accurate and sensitive enough. Thus,as skin is the largest organ of the human body, even if weak enzymatic activities are observed, they can become consequent when considering its total surface area. Consequently, research on skin metabolism would require a real scientific effort and dynamism to characterize skin metabolizing enzymes and their activities.
In addition, the 7th European amendment to the cosmetic directive forbids the use of animal testing to assess the effectiveness and safety of new cosmetics. This policy has forced the cosmetic industry to develop in vitro tools as alternative methods to animal experiments. Reconstructed human skin models are a part of them. For these reasons, these models have to be characterized and compared with normal human skin in terms of metabolic capabilities.
This work presents a review of the L'Oreal research strategy and main results in the characterization of skin metabolic equipment and its catalytic capabilities. Thus, characterization for the expression of several enzymes (CYP450, Esterase, NAT, GST, UGT, SULT…) and their catalytic activities (Apparent Km, Vmax and clearance) in various reconstructed skins were compared to normal human skin samples.

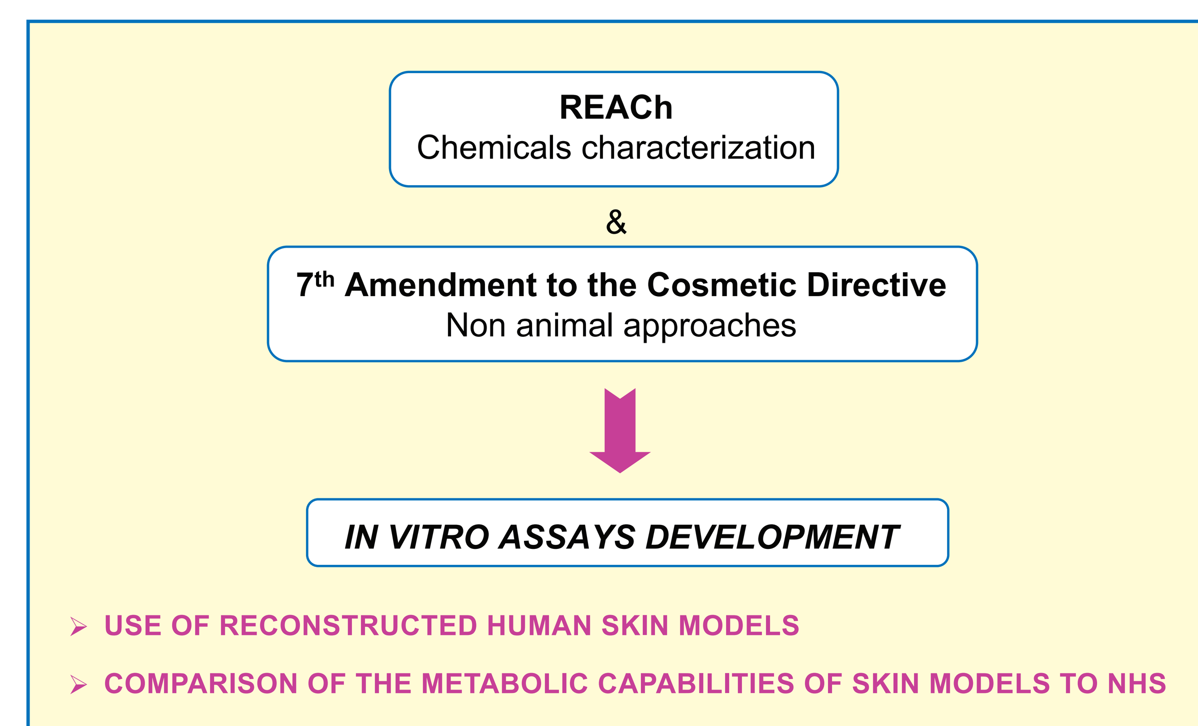
INTRODUCTION

1. LIVER & SKIN

	LIVER	SKIN
		VS
Main cell type	Hepatocytes	Keratinocytes
Main functions	Drug metabolism	Physical barrier, thermoregulation…
Weight	2% body weight, 1.5 kg	15% body weight, 2 m²

➢ CHARACTERIZATION OF THE SKIN METABOLIC CAPABILITIES

2. REGULATORY CONTEXT

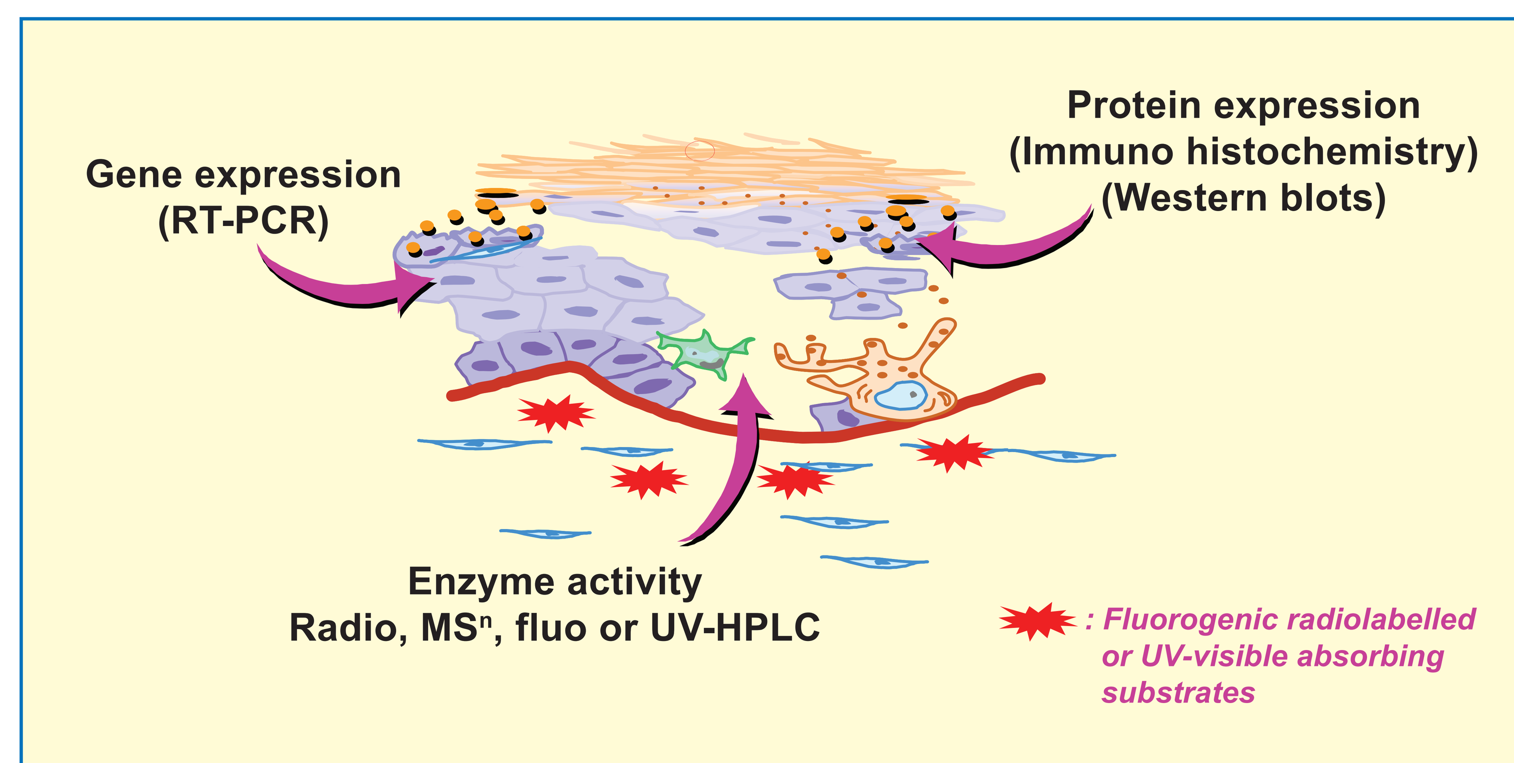


➢ USE OF RECONSTRUCTED HUMAN SKIN MODELS

➢ COMPARISON OF THE METABOLIC CAPABILITIES OF SKIN MODELS TO NHS

MATERIALS AND METHODS

1. DEVELOPPED APPROACH



2. RECONSTRUCTED HUMAN SKIN MODELS

SkinEthic laboratories

TYPE	ORIGIN	KITS
Normal Human Skin (NHS): *Epidermis/Dermis*	• Mammoplasties	**variable** ∅: ~1.2 cm S: ~1.1 cm²
Episkin™: *Reconstructed epidermis*	• NHK (mammoplasties) • Pool 4 – 5 donors Support: BPER	**12 inserts/kit** ∅: ~1.2 cm S: ~1.1 cm²
SkinEthic™ RHE: *Reconstructed epidermis*	• NHK (foreskin/abdoplasties) • 1 donor/Pool 2 donors Support: Polycarbonate	**24 inserts/kit** ∅: ~0.8 cm S: ~0.5 cm²
Full thickness of Episkin™: *Reconstructed epidermis / equivalent dermis* FTM	• NHK (mammoplasties) • Pool 4 – 5 donors Support: Polycarbonate	**6 inserts/kit** ∅: ~1.8 cm S: ~2.5 cm²

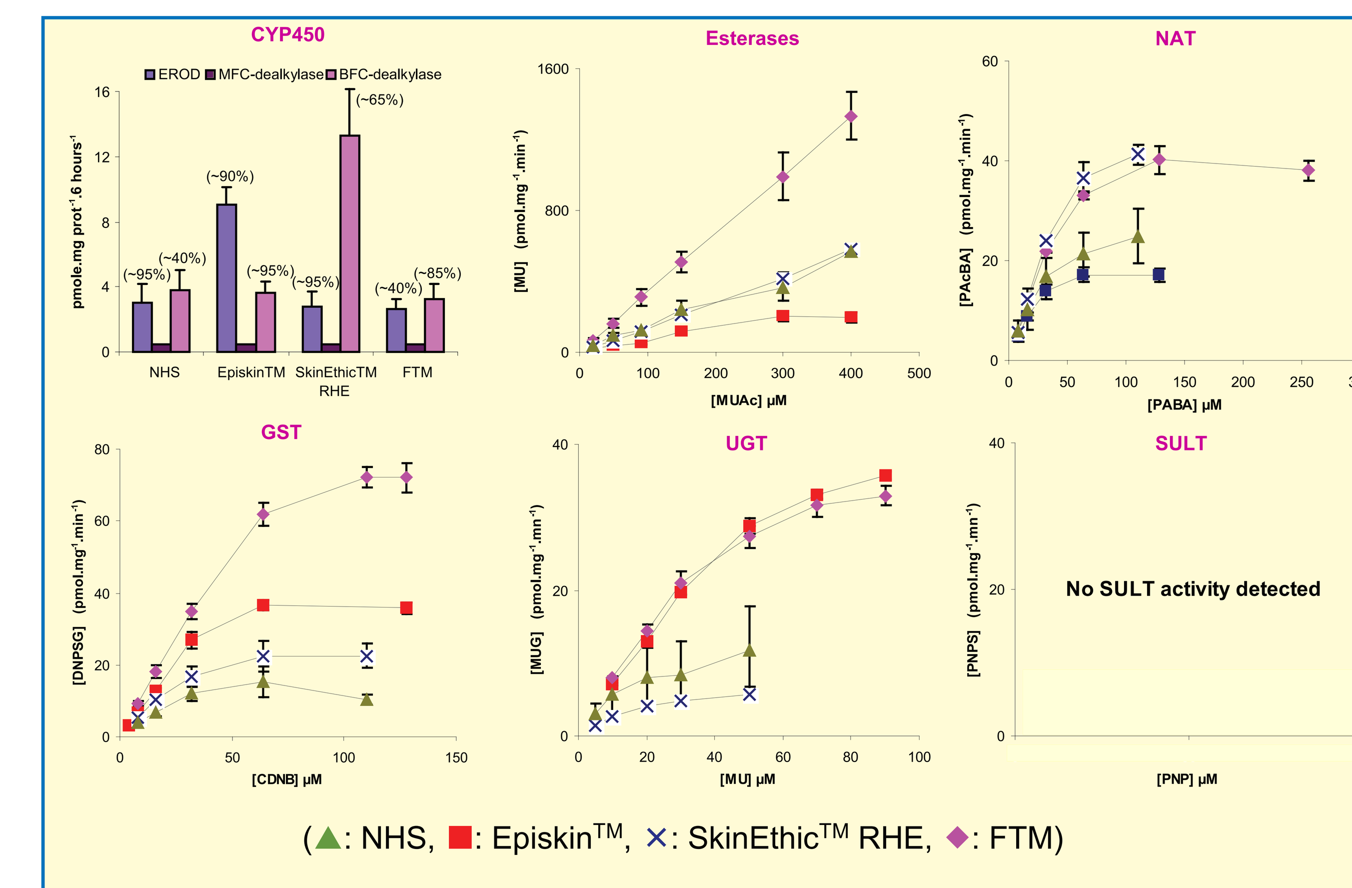
RESULTS

1. mRNA EXPRESSION

Enzymes	Isoformes	NHS		RECONSTRUCTED HUMAN SKIN MODELS		
		Epidermis	Dermis	Episkin™	FTM	SkinEthic™ RHE
CYP450	1A1	+	(+)	-	-	-
	1A2	(+)	-	-	-	-
	1B1	(+)	**+**	(+)	(+)	+
	2B6	(+)	+	-	(+)	-
	2C18	(+)	-	-	+	+
	2C19	nd	nd	-	nd	+
	2D6	(+)	(+)	(+)	(+)	(+)
	2E1	(+)	(+)	(+)	+	-
	3A5	(+)	-	(+)	(+)	**++**
	3A7	(+)	-	-	-	-
Esterases	AADAC	nd	nd	++	nd	++
	CEL	nd	nd	(+)	nd	(+)
	ESD	nd	nd	++	nd	++
	CES1	nd	nd	++	nd	++
	CES2	nd	nd	++	nd	++
	ACHE	nd	nd	-	nd	-
	PLA2G4B	nd	nd	++	nd	++
NAT	NAT1	(+)	(+)	++	(+)	++
	NAT2	-	-	-	-	-
	NAT5	++	++	++	++	++
GST	GSTA3	++	(+)	-	nd	(+)
	GSTA4	nd	nd	++	nd	++
	GSTM2	++	++	(+)	nd	++
	GSTM3	++	++	(+)	nd	(+)
	GSTM5	(+)	++	nd	-	(+)
	GSTP1	+++	+++	++++	++++	+++
	GSTT1	++	++	++	++	++
	GSTZ1	nd	nd	++	nd	++
UGT	UGT1A1	nd	nd	-	nd	-
	UGT1A3	nd	nd	++	nd	++
	UGT1A4	nd	nd	(+)	nd	(+)
	UGT1A5	nd	nd	++	nd	++
	UGT1A6	++	(+)	++	nd	++
	UGT1A7	nd	nd	++	nd	++
	UGT1A8	nd	nd	++	nd	++
	UGT1A9	nd	nd	-	nd	-
	UGT1A10	(+)	(+)	++	(+)	++
	UGT2B17	-	(+)	-	-	-
	UGT2B28	-	(+)	-	-	-
SULT	SULT1A1	++	++	(+)	++	(+)
	SULT1E1	(+)	(+)	++	++	++
	SULT2A1	-	-	-	-	-
	SULT4A1	nd	nd	-	nd	(+)
	SULT2B1	+++	++	+++	+++	+++

- < 1000 copies/µg total ARN, **(+)** > 1000 copies/µg total ARN, **+** > 10000 copies/µg total ARN,
++ > 100000 copies/µg total ARN, **+++** > 1000000 copies/µg total ARN, **++++** > 5000000 copies/µg total ARN

2. MEASUREMENT OF CATALYTIC ACTIVITIES



DISCUSSION AND CONCLUSION

1. ENZYME ACTIVITIES

- ❑ Low basal expression and activity of CYP450 involved in «Drug metabolism» (!! induction !!)
- ❑ High esterase activity (Low affinity with the compound used as substrate)

- ❑ NAT activity was detected
- ❑ GST activity was detected
- ❑ UGT activity was detected
- ❑ Very weak SULT activity except for steroid sulfation

- ❑ Other enzymes to be quickly tested:
  - Phase I: COX/ALOX (peroxidases) & ADH/ALDH (in progress)
  - Phase II: COMT

2. RECONSTRUCTED MODELS & HUMAN SKIN COMPARISON

Apparent enzymatic parameters were calculated and compared between reconstructed skin models and normal human skin:
- ➢ Affinity (Km) and Maximal velocity (Vmax) are different
- ➢ Clearances (Vmax/Km) are often similar

➢ **SKIN IS RATHER A DETOXIFICATION ORGAN THAN A BIO ACTIVATING ONE**

➢ TOXICITY APPEARS IN SKIN WHEN:
- DETOXIFICATION SYSTEMS ARE OVER EXPOSED TO TOXICANTS
- REACTIVE MOLECULES ARE RELEASED IN LARGE AMOUNTS

➢ RECONSTRUCTED HUMAN SKIN MODELS ARE GOOD ENOUGH PREDICTIVE TOOLS OF SKIN METABOLISM AND TOXICITY

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