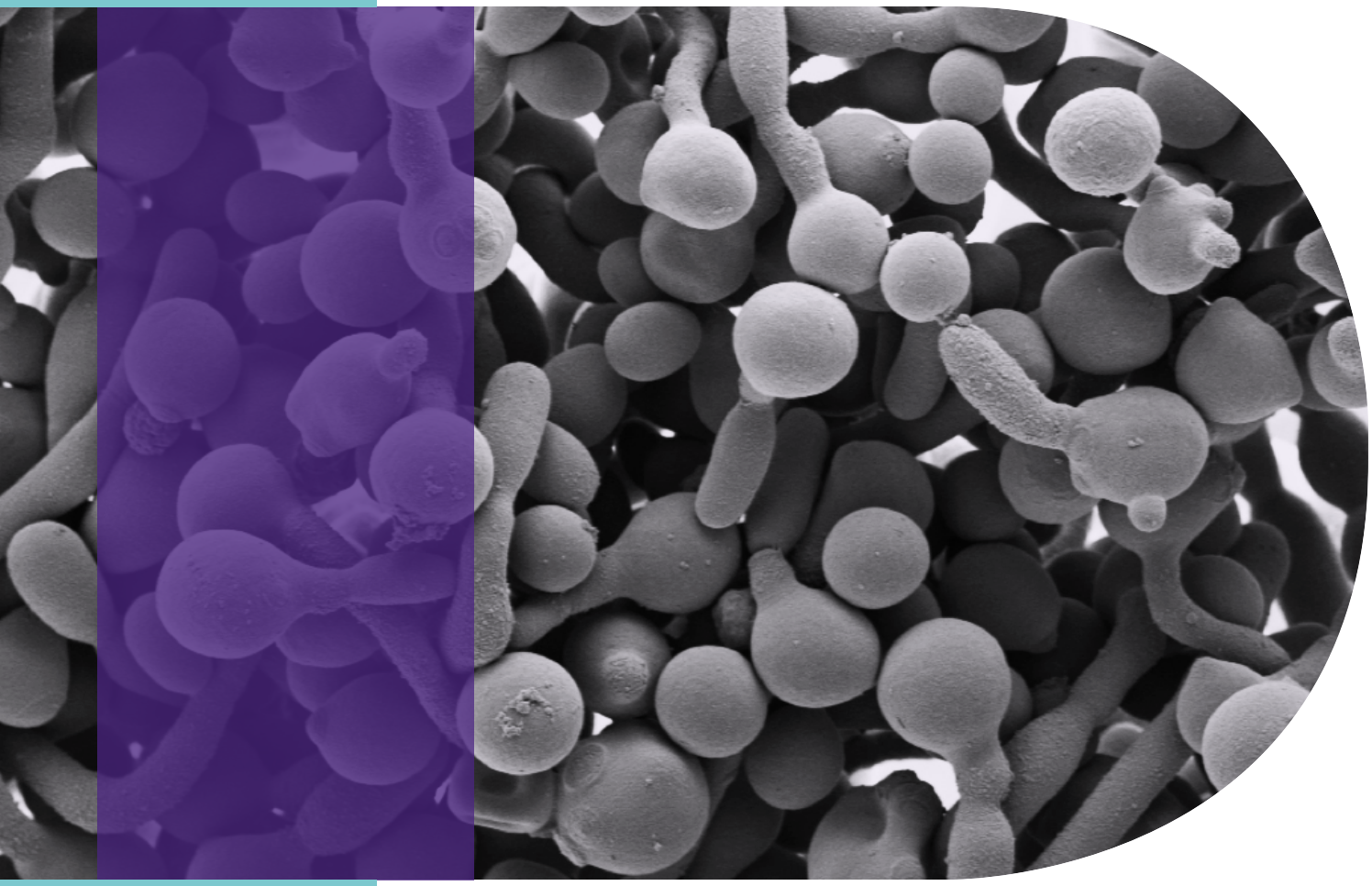
Nutritionals



Lactobacilli spp. on intestinal models

VitroScreen
Leading Innovation in Pre-Clinical Testing

VitroScreen's expertise to support FOOD SUPPLEMENTS' functionality

VitroScreen has developed a precise, sensitive and quantitative approach to define the mechanisms of action and functionality of Nutritionals under realistic exposure conditions on body barriers and body organs: Food Supplements pre-clinical studies become thus reliable, fast and clinically relevant.

The innovative experimental approach provides sound scientific evidences and it is applicable to:

- R&D projects
- Dose finding
- Nutrients, associations of Nutrients and formulation screening
- Advanced delivery technologies
- Patent application
- Scientific communication to health care professionals
- Marketing
- The design of further clinical investigations based on realistic human exposures
- Proof of concept for EFSA health claims.

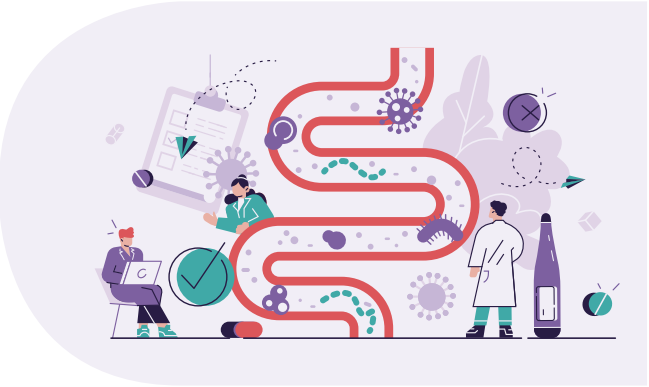
***In Vitro* protocols developed by VitroScreen on 3D Human tissues and miniaturized Organs are UNIQUE, EVIDENCE-BASED opportunities to reach the required evidences of Food Supplements' functionality.**

How to mimic *in vitro* the complexity of the whole process?

VitroScreen proposes to investigate Food Supplements' functionality in a **2 steps approach**:

- The intestine is the first biological barrier interacting with Nutritionals. It is fundamental to define the relevant dose with a potential biological activity. Intestinal passage, transport and absorption are quantified on well established **INTESTINAL BARRIER MODELS.**

- Nutritionals and nutrients after systemic absorption reach body's target organs. **3D HUMAN MINIATURIZED ORGANS and TISSUES** represent advanced *in vitro* systems to explore Food Supplements' biological properties and mechanisms of action.



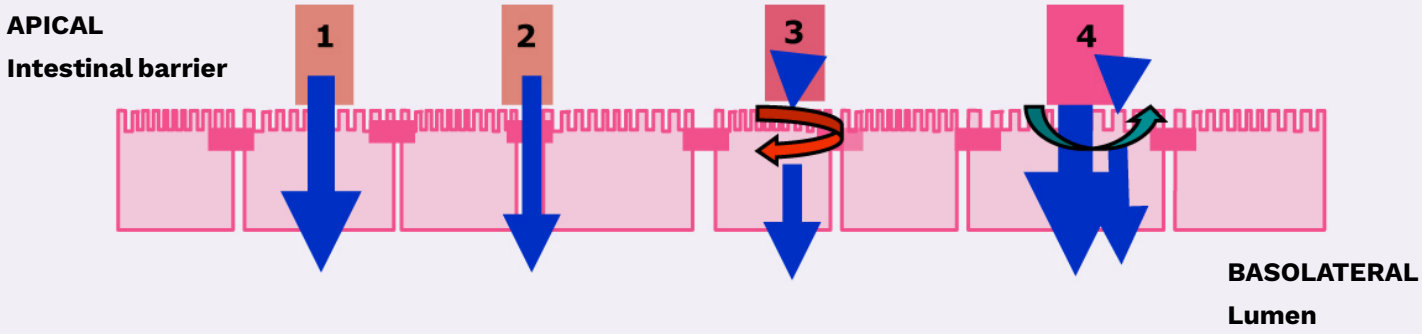
INTESTINAL PASSAGE, TRANSPORT AND ABSORPTION

The intestinal barrier is the guarantee of protection of the whole organism against toxic substances but also the limiting factor of Nutritionals' absorption.

Food Supplements' bio-availability and digestion are influenced by their physicochemical properties, pH specific intestinal *milieux* and the microbiota. Intestinal passage results allow to estimate a range of blood concentrations applicable to *in vitro* efficacy study doses.

Intestinal penetration and absorption to define transport mechanisms: active or passive

- Study design and analytical method's validation on Caco-2 based models
- Small intestine spheroids and colon organoids, customized models VitroScreen



1 + 2 = PASSIVE TRANSPORT | 3 = ACTIVE TRANSPORT | 4 = EFFLUX

Food supplements' efficacy on miniaturized organs

Spheroids and organoides reproduce *in vitro* the organs that Nutritionals are intended to reach, thus covering Food Supplements' functionality and mechanisms of action on almost all body organs.

By mimicking the *in vivo* biological environments and adopting realistic and clinically relevant human exposure conditions, the robustness of the *in vitro* experimental design and its predictivity are guaranteed on multiple targets: small intestine, colon, liver, pancreas, bladder, prostate, cartilage, skin and dermopapilla, adipose tissue, mucosae, eye, airways.

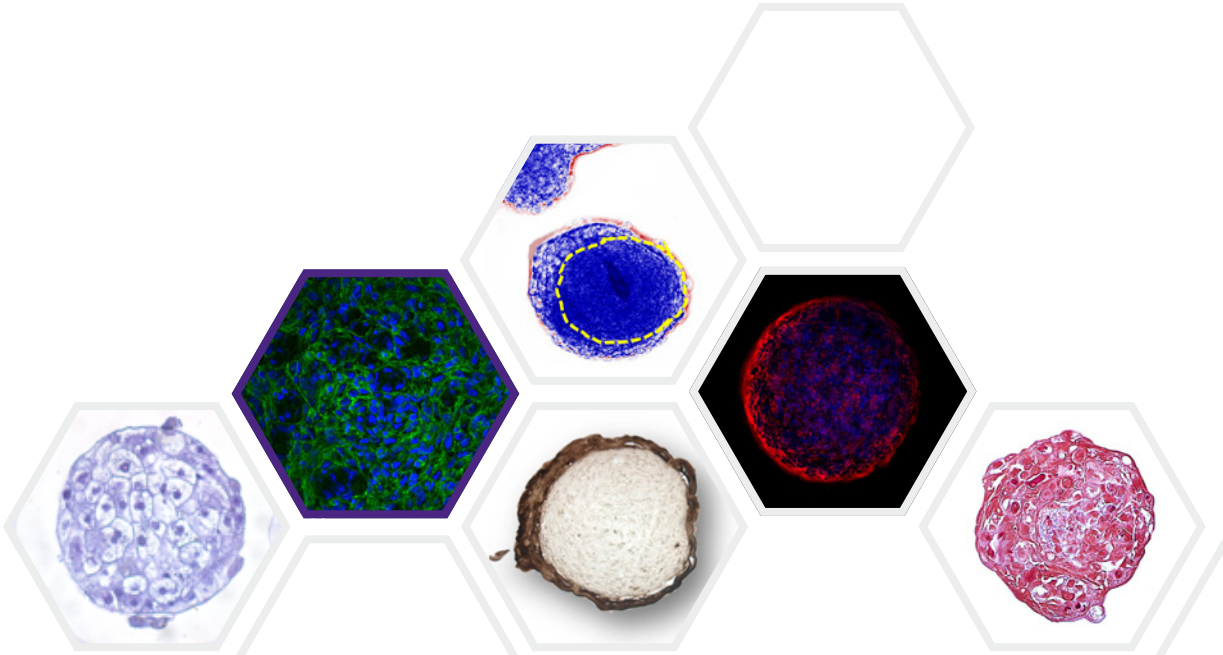
VitroScreen ORA
Organoids Research Applications

VitroScreen platform includes tissue specific 3D miniaturized organs mirroring phenotypic characteristics of the donor's cells and related metabolic pathways.

We develop standard and customized models using primary cells or Human IPSC-derived MSCs cultivated as single type or in co-colture.

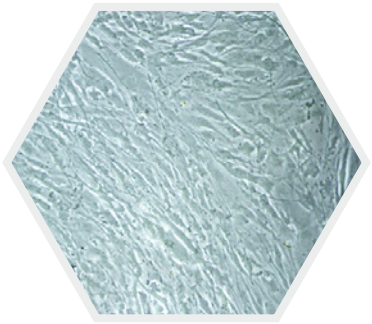
Each spheroid reproduces a microenvironment where extra cellular matrix deposition, organization and dynamic evolution occur naturally, relying on an efficient cells-matrix interaction.

VitroScreen models are suitable to perform long-term experimental protocols (up to 28 days) and can be cultivated under homeostatic or stress/disease conditions with treatment doses closer to *in vivo* ranges.

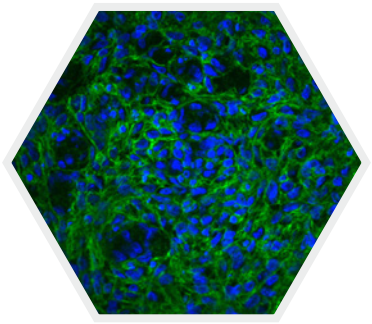


Miniaturized body organs: ADVANTAGES and BIOLOGICAL RELEVANCE

2D cell culture



3D scaffold-free spheroids engineering



Miniaturized Organs

- Low functionality
- Flat
- Cell density ca. 0.1% of tissue
- Dilution of all secreted factors
- Poor cell to cell contacts

- Bio-mimetic
- Polarized
- No scaffold, maximal cells-matrix interactions
- 3D cell contacts resulting in high tissue density
- Co-culture of cell types
- Homeostatic condition
- Efficient ECM production
- Spatial distribution and organ-like functionality

NUTRITIONAL AREAS

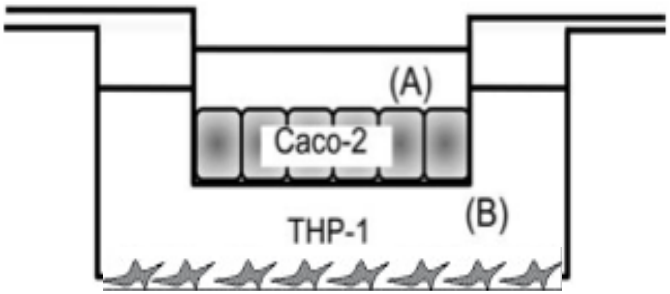
FUNCTIONALITY and MECHANISM OF ACTION	MINIATURIZED ORGANS
INTESTINAL AND DIGESTIVE HEALTH	INTESTINAL SPHEROIDS
LIVER METABOLIC (LIPIDS ACCUMULATION, HYPER-CHOLESTEROLEMIA, STEATOSIS, ENERGY)	LIVER SPHEROIDS
PANCREAS and ADIPOSE TISSUES: GLUCOSE UPTAKE, LIPIDS ACCUMULATION and WEIGHT BALANCE	PANCREATIC ISLETS AND ADIPOSE TISSUE SPHEROIDS
CELLULAR ENERGY METABOLISM and FITNESS	ALL MINIATURIZED MODELS
CARTILAGE-JOINTS	CARTILAGE SPHEROIDS
UROGENITAL TRACT	BLADDER, PROSTATE SPHEROIDS, VAGINAL MUCOSA STROMA
HEALTH and BEAUTY FROM WITHIN	**3D RECONSTRUCTED BODY BARRIERS**
MUCOSAE: ORAL, RESPIRATORY and VAGINAL TRACT	VIABLE in homeostatic conditions and COLONIZED with site specific MICROBIOTA
EYE, SKIN, HAIR FOLLICLE, NAILS	
BODY BARRIER-GUT AXIS for new generation products	INSIDE-OUT BEAUTY Double efficacy based on systemic and topical exposure

INTESTINAL BARRIER PROTECTION IBD and LEAKY GUT

Caco-2, Caco-Goblets	Film forming, boosting barrier function structure, muco-adhesion, permeability, anti-oxidant properties, apoptosis induction to mimic gas production, lactose tolerance
	Multiple endpoint analysis approach to evaluate barrier integrity and modified permeability: • Bacteria viable counts • TEER measurement • Tight junction proteins localization (IF) • LY passage
VitroScreen ORA **SMALL INTESTINE AND COLON**	**Advanced Customized Models**: CO-COLTURE WITH THP-1 CELLS • Leaky Gut syndrome based on Glutamine deprivation • Inflammation induced by cytokines mix • Immuno-mediated Inflammatory Bowel Disease (IBD)

IMMUNO-COMPETENT GUT

IMMUNO COMPETENT GUT: Caco-2 + THP-1 monocytes



PRE-PRO-POST BIOTICS
Bacterial adhesion, growth, survival, interaction and cross talk with intestinal cells
Immuno-mediated bacterial response
Anti-inflammatory properties
Immuno-modulatory efficacy

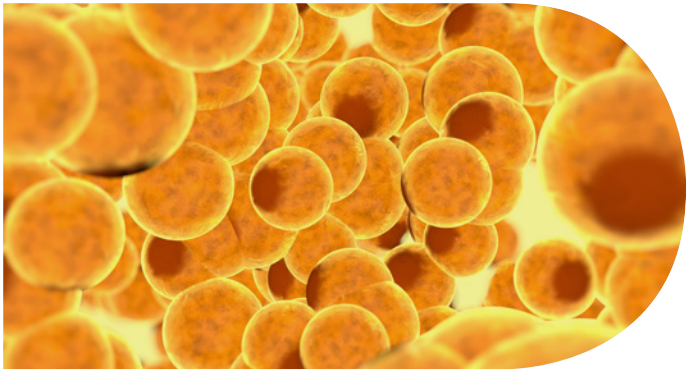
COMPETITION with PATHOGENS
Competitive adhesion to the intestinal mucosa
Competition with bacterial «secretome»

Intestinal pathogen invasion model:
E. coli (α-ACTININ by HIC) and microvilli ultrastructure by SEM

Customized-strain specific models

LIVER and PANCREAS

3D Insight™ Human Liver Microtissues	**Hepatotoxicity** Acute and repeated exposures: MEA approach and IF by high content imaging
	Metabolic competence **Cytochrome P450** activation/induction with validated references
	Fatty acid oxidation (FAO) & mitochondrial dysfunction Based on oleate and oxygen consumption to monitor β-oxidation, FA specific transporters (CPT1 and CPT2), ATP and glycolysis as complementary read-outs
3D Insight™ Human Liver Microtissues	**Liver Steatosis** Triglyceride quantification and lipid accumulation by bioluminescence and IF high content imaging on live cells, pro-inflammatory biomarkers (PPAR-α) Oxidative stress: ROS and GSH/GSSG quantification Energy and cellular metabolism Quantification of GLUTAMINE/GLUTAMATE, LACTATE
VitroScreen CRA **LIVER FH spheroids**	**Hypercholesterolaemia** Quantification of CHOLESTEROL and CHOLESTEROL ESTERS MGCR (HMG-CoA reductase) as a rate limiting enzyme of cholesterol synthesis SOAT1 (ACAT), Sterol O-Acyltransferase I catalyzes the formation of fatty acid – cholesterol esters; CYP7A1 (cytochrome P450 Family 7 Subfamily A) Oxidative stress: ROS and GSH/GSSG quantification
Pancreatic Islets	**Diabetes II feature** GLUCOSE UPTAKE and INTAKE Insuline resistance (customized) Lipolysis: GLYCEROL and TRYGLICERIDES quantification Oxidative stress: ROS and GSH/GSSG quantification



WEIGHT BALANCE, CELLULAR METABOLISM and FITNESS

	Energy and cellular metabolism based on ATP
VitroScreen ORA ADIPE	Adipocytes differentiation, lipids accumulation, metabolism and destock, inflammatory pathways investigation, influence of hormons qRT- PCR and high content 3D imaging analysis on relevant gene signatures
• Primary Human Subcutaneus Pre-adipocytes • Human IPSC-derived MSCs	• Metabolic studies and weight balance management • Metabolic disorders simulation and long-term exposures • Stress models: inflammation, differentiation and fibrosis • Lipolysis, adipogenesis, lipo-boosting, lipid destock: slimming, plumpling, resculpting Innovative and advanced model to investigate bacterial secretome mechanims of action (customized models)

CARTILAGE & JOINTS

VitroScreen ORA
CARTILAGE

On human primary cells , phenotypical features of native cartilage suitable to assess:

Chondro-protective efficacy against hyaluronic acid degradation (anti-jaluronidase) and promoting collagen II deposition
Boosting of earlier differentiation and metabolic activation leading to a chondrogenic profile in favour of jaluronic acid deposition
Metabolic studies: it expresses key biomarkers of tissue differentiation

- Inflammatory model mimicking osteoarthritis
- Cartilage degradation is quantified targeting Aggrecan, collagen II, Hyaluronan synthase, Hyaluronidase, S 100- involved in tissue regenerative capacity

Health and beauty from within

FOOD SUPPLEMENTS developed to reach BODY BARRIERS in specific areas to determine beneficial health effects from inside (eye and skin, uro-genital tract, mouth and throat, airways) can be assessed for their direct efficacy:

- in strengthening barrier protective function
- in fighting pathogens invasion
- in boosting innate immunity

In vitro Systemic exposure mimicks daily nutritionals intake

GASTRO-INTESTINAL TRACT: ORAL and OESOPHAGEAL MUCOSAE

3D reconstructed human tissue models	
Oral Epithelium	Film forming properties, enhancing barrier protection properties (long-term exposure) Muco-adhesion and persistency with/without reconstructed saliva Bio-availability, absorption and penetration kinetics
Oesophageal Epithelium	Film forming and bio-adhesive properties, H+ block and buffering properties
	GERD model based on **Simulated Gastric Fluid** (SGS, US Pharmacopeia): preventive and repairing efficacy
	Multiple Endpoint Approach: Tight junction proteins localization (IF) Claudin-1/4 and Mucins LY passage

UROGENITAL TRACT

3D reconstructed Vaginal Epithelium	*Lactobacilli* sp adhesion, viability and growth, efficacy on epithelial differentiation, innate immunity response, moisturization and decongestant properties Antimycotic efficacy, competition model based on *Candida Albicans* and pathogens *Lactobacilli* sp co-colonization with pro-biotics
3D reconstructed Bladder Epithelium	Protection of barrier integrity, muco-adhesion and film forming Prevention of bacteria adhesion, protection against bacteria damage
VitroScreen ORA **VAGINAL MUCOSA STROMA**	Bio-revitalizing and hydrating properties, new ECM production counteracting mucosal atrophy
VitroScreen ORA **PROSTATE** HPrECs and PrSMCs: Human Primary Prostate Epithelial Cells and Prostate Human Smooth Muscle Cells. Co-culture	It closely mimics the prostatic capsule: a core of epithelial secretory cells embedded in a thin layer of smooth muscle cells forming fibromuscular bands. 5-α reductase model: TST conversion in DHA Suitable for absorption and metabolism studies

SKIN, NAILS, HAIR

SKIN, NAILS, HAIR

Full Thickness Skin	**Wound healing:** Dynamic monitoring during 2 weeks of key events of re-epithelization and wound healing processes
	Biofilm on infected wounds **Diabetic ulcers** (glycation): key events of re-epithelization and wound healing processes Advanced model including pathogens
3D Reconstructed Human Epidermis	**Inflammasome** and oxidative stress model NfkB translocation, NLRP3 pathway, activated caspase-1, IL-1 beta
	Atopic dermatitis RHE colonized with *S.aureus* + THP-1 cells in co-culture **Immuno-competent-AD**: reduced filaggrin expression, inflammatory pathway based on TSLP activation, TNF-α and TLRs, biofilm formation
	Psoriasis and **Psoriatic plaques** induced by cytokines mix in systemic or local exposure
	Antimycotic efficacy The model is based on reconstructed human epidermis (RHE) infected with the fungus that acts as a support for a nail obtained from bovine donors. Suitable to evaluate the efficacy of market references in healing the infected nail and cuticula based on GMS staining

SKIN EXPOSED TO UV RADIATION

3D Reconstructed Human Pigmented Epidermis Full Thickness Skin	DNA damage and repair based on biologically relevant UV doses: acute *vs* repeated exposures and assessment of delayed damages and /or physiological damages recovery
3D Reconstructed Human Epidermis 3D Reconstructed Human Pigmented Epidermis	Oxidative stress and advanced photoprotection: UV early and delayed damages on multiple targets and customized protocols

SKIN and HAIR

3D Reconstructed Human Epidermis Full Thickness Skin VitroScreen ORA DERMIS	Skin firmness: counteracting atrophy and ageing Efficacy model for aged skin biorevitalization Deep moisturization Daily environmental stresses protection: oxidative stress, hypoxia, pollution, dryness
VitroScreen ORA DERMOPAPILLA PATENT IT n. WO 2019/092667	**Unique *in vitro* model to study:** Hair growth Hair cycling, anagen/catagen transition and suitable to follow catagen involution Anti-hair loss efficacy Reliable anagen metabolic phase to investigate hair growth on long-term treatment

EYE HEALTH

3D reconstructed Human Corneal Epithelium	DRY EYE DISEASE (DED) Dynamic monitoring of molecular and morphological modifications of corneal epithelium and the efficacy of treatments in the physiological recovery from dryness based on 3 damage levels
	Immuno-competent DED: THP-1 cells in co-culture Innovative and clinically relevant model for a deeper investigation of ocular, surface barrier impairment, inflammation and immuno-activation
	OCULAR SURFACE BARRIER PROTECTION against EXPOSOME (UVs, Blue Light, Particulate Matter, Microbiome)
VitroScreen ORA STROMA HKs: Primary Human Keratocytes	3D scaffold free spheroids produced with primary keratocyte cells allowing to investigate ECM formation, assembly and re-modelling It reproduces corneal stroma ECM modifications induced by pathologies or injuries (keratoconus, keratitis)

RESPIRATORY TRACT: NASAL and BRONCHIAL EPITHELIA

3D reconstructed epithelia	Co-culture with THP-1 cells: immuno-mediated mechanisms Advanced model to identify: • Innate immunity boosting mechanisms • Detoxifying mechanisms • Probiotics adhesion and growth • Antibacterial and antiviral efficacy

The regulatory framework

An increasing number of foods and food supplements sold in Europe bears health claims. Since 2006 the EC Regulation 1924/2006 requires that any health claim made on food be scientifically justified in order to protect consumers from inaccurate or misleading information.

Human clinical studies are crucial to substantiate the claim. However, other studies can provide useful supporting evidences: for example on the mechanism of action *(EFSA Journal 2011;9(4):2135)*.

The results and the quality of the studies presented, including human, animal, *in vitro* and mechanistic studies and the plausibility of the claim itself are weighed by EFSA.

On the base of EFSA's opinion the European Commission and the Member States decide whether or not authorize the claim.

***In Vitro* protocols developped by VitroScreen on 3D Human tissues and miniaturized Organs are UNIQUE, EVIDENCE-BASED opportunities to reach the required evidences of Food Supplements functionality.**

VitroScreen
Leading Innovation in Pre-Clinical Testing

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