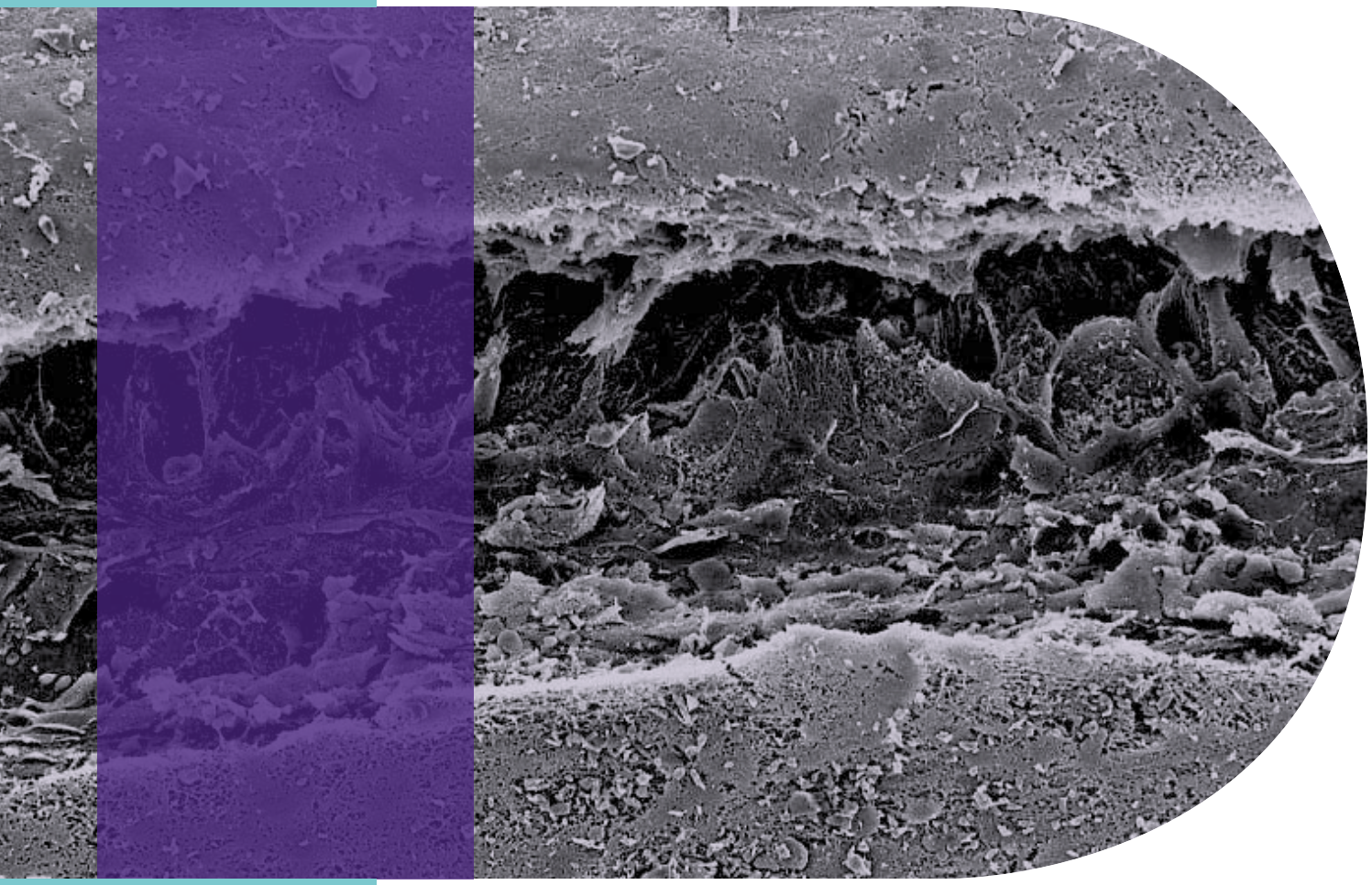
Pharmaceuticals



Corneal re-epithelization on HCE (SEM)

VitroScreen
Leading Innovation in Pre-Clinical Testing

VitroScreen proposes to adopt an ethical, closer-to-humans and clinically-relevant pre-clinical testing strategy.
We aim to foster innovation in R&D processes at different steps by using 3D reconstructed human tissues and spheroids models that are more predictive for human exposure and metabolic responses.

PRE-CLINICAL EFFICACY AND MECHANISM OF ACTION

VitroScreen has developed standard ready-to-use experimental models and high content customized models applicable to drug discovery (medium throughput screening), dose finding, prototype and target validation covering a large set of industrial needs in different therapeutic areas before performing clinical trials.

Experimental protocols to assess Efficacy and Mode of Action (MoA) are based on Multiple Endpoint Analysis (MEA) approach on 3D human tissue models allowing deeper investigation and quantification of validated standard and customized parameters and biomarkers.

Dermatology

3D Reconstructed Human Epidermis (RHE), Full Thickness Skin (FT-Skin), VitroScreen DERMIS

Skin barrier development and modifications	Epidermal differentiation, Tight Junctions (TJs) structure, Kc proliferation, apoptosis, innate immunity, barrier permeability
Wound healing	Dynamic monitoring over 2 weeks of key events of re-epithelization and wound healing processes
Biofilm formation on infected wounds, Diabetic ulcers	Specialization model including pathogens and glycation
Inflammasome	Mechanical and physical stress; key biomarkers up to 72h: NfkB translocation, NLRP3 pathway, activated caspase-1, IL-1β
Acne lesions with/without *C.acnes*	Relevant gene signature: MMP-13, IL-8, Decorin
Skin atrophy	Fast and predictive experimental window to assess FANS index; it can be used as efficacy model for aged skin
Atopic dermatitis: RHE colonized with *S.aureus* + THP-1 cells in co-culture	Immuno-Competent AD: inflammatory pathway based on TSLP activation, TNF-α and TLRs, biofilm formation, filaggrin down regulation
Psoriasis and Psoriatic plaques	Inflammation induced by cytokines mix in systemic or local exposure: fast and reproducible model suitable for ingredients screening and MoA
Filler efficacy and tolerance	It mimicks clinical procedure targeting papillar dermis: dynamic monitoring of bio-revitalization and MoA

Photo-Dermatology

3D Reconstructed Human Epidermis (RHE) and BALB/3T3

Phototoxicity	Validated OECD TG 432. Protocol on RHE in case of not concluding results / not applicability / formulations
UV activation and melanogenesis	Applicable to investigate UV biological response on Kc and melanocytes; early screening of UV-induced sensitization potential; MoA of actives and formulations
DNA damage and repair	Based on biologically relevant UV doses: acute *vs* repeated exposures and assessment of delayed damages and / or physiological recovery from damages
Oxidative stress	UV radiations or Buthionine Sulfoximine (BSO) induced: early and delayed damages. Customized protocols

Orthopedy

VitroScreen CRA **CARTILAGE**	3D scaffold free spheroids produced with primary Human Chondrocytes (HCs) developed by the *In Vitro* Innovation Center. It recapitulates phenotypical features of native cartilage Metabolic studies: it expresses key biomarkers of tissue differentiation Cartilage degradation and regeneration Inflammatory model

Ophthalmology

3D Reconstructed Human Corneal Epithelium (HCE) and VitroScreen CORNEAL STROMA

Dry Eye Disease (DED)	Model published in 2001: it allows the 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
DED: Ultrastructure analysis of Microvilli network	By SEM
Immuno-competent DED: THP-1 cells in co-culture	Innovative model for a deeper investigation of ocular surface barrier impairment, inflammation and immuno-activation
Corneal wounds	A standardized mechanical injury determining the establishment of the key events of corneal wound: migration, inflammation, proliferation, ECM remodeling and re-epithelization
Ocular surface barrier protection against Exposome (uvs, blue light, particulate matter, microbiome)	Epithelial cells response with mechanism-related biomarkers: customized protocols within the *In Vitro* Innovation Center
Hyper-osmolarity and inflammation	Reproducible induction of inflammatory response: it allows to assess early decongestant efficacy
VitroScreen CORNEAL STROMA	3D scaffold free spheroids produced with primary keratocyte cells allowing to investigate ECM formation, assembly and re-modelling. High content imaging and customized models with cells from different donors (health and pathologies)
Eye Tolerance: applicable to formulations and ingredients	To investigate direct toxicity mechanisms and recovery from damage after acute and repeated exposures

Respiratory Tract

3D reconstructed human nasal and bronchial epithelia

Epithelial barrier integrity and protection against exposome (particulate matter, sensitizers, pollens, pathogens, viruses: adhesion and defence mechanisms)	Experimental protocols and read-outs have been established to respect the peculiarity of airway epithelium physiology: TEER, LY, TJs structure. Advanced models have been developed by the *In Vitro* Innovation Center coupled with GARD®air to monitor epithelial responses and investigate mechanisms related to recovery from damages and detoxifying properties
Inflammation and oxidative stress, calcium channels	Induced by environmental stresses: customized protocols developped by the *In Vitro* Innovation Center
Healing and barrier function strengthening	Suitable to follow epithelial healing process and TJs structuring during 3 days with adapted positive controls to identify the mechanims of action
Phagocytosis and cilia ultrastructure	To quantify epithelial phagocytosis efficiency coupled with SEM analysis for cilia ultrastructure
Decongestant efficacy	Suitable to quantify isotonic and hypertonic solutions efficacy in restoring epithelial homeostasis
Inflammation and immuno-mediated response: co-culture with THP-1 cells	Advanced screening model to identify potential sensitizers and detoxifying mechanisms
Host response to commensal and pathogens Disrupting established biofilm/preventing biofilm formation	Colonized tissue models developped by the *In Vitro* Innovation Center
Probiotics adhesion and innate immunity boosting	Acute and repeated exposure with validated biomarkers

Gynecology

VitroScreen ORA **ENDOMETRIUM** VitroScreen ORA **VAGINAL MUCOSA STROMA**	3D scaffold free spheroids developed by the *In Vitro* Innovation Center
3D Reconstructed Vaginal Epithelium	*Lactobacilli sp* adhesion, epithelial differentiation, innate immunity response, moisturization and decongestant properties, antimicotic efficacy, competition model based on *Candida Albicans* and Lactobacilli *sp* co-colonization. Suitable to assess MoA of ingredients and formulations: systemic and topical exposures

Urology

VitroScreen ORA **PROSTATE**	It closely mimicks 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 passage, penetration and metabolism studies
3D Reconstructed Bladder Epithelium	Protection of barrier integrity, muco-adhesion and film forming, prevention of bacteria adhesion, protection against bacteria damage, decongestant activity. Suitable to assess MoA of ingredients and formulations: systemic and topical exposures

Gastroenterology

3D reconstructed human oral, oesophagous, colorectal epithelia, intestinal models

Immuno-competent gut and IBD	Advanced model based on a Caco-2 and THP-1 monocytes co-colture Inflammation induced by cytokines mix Monocyte epithelial adhesion assay (IF) Immunomodulatory activity of prebiotics and probiotics Suitable to investigate immunomediated anti-bacterial response and anti-inflammatory properties
Leaky gut	Advanced model on Caco-2 based on glutamine deprivation: it is suitable for a dynamic approach of preventive and protective efficacy, anti-oxidant properties
Film forming activity, TJS boosting, enhancing the barrier protection properties, muco-adhesion, permeability	• TEER measurement • TJ proteins expression (RT-PCR) • TJ proteins localization (IF) • LY passage
Inflammation model by systemic exposure to SCFA	It mimicks gas production related mechanism: apoptosis
Intestinal pathogen invasion model: *E. coli* customized models (strain specific)	Bacterial adhesion: α-actinin by HIC and microvilli ultrastructure by SEM MEA to evaluate barrier integrity and modified permeability including bacterial counts
VitroSceen ORA® - INTESTINE	Under development by the *In Vitro* Innovation Center

Liver Diseases

3D Insight™ Human Liver Microtissues and VitroScreen ORA customized spheroids

Hepatotoxicity	Acute and repeated exposures: MEA approach and IF by high content imaging
Metabolic competence: CYP 450	Cytochrome 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-out
Hypercholesterolemia model	VitroScreen ORA: advanced customized model
Steatosis, non-alcoholic Steatohepatitis (NASH)	Triglyceride quantification, lipid accumulation; lipid HIC and IF by high content imaging on live cells. Pro-inflammatory biomarkers (PPAR-α)

Safety and Tolerance

- In line with the Company's mission and expertise, also supported by regulatory and ethical requirements, VitroScreen has established standard protocols for safety assessment based on realistic and predictive exposure protocols (i.e. doses, acute or repeated exposures) on *in vitro 3D* reconstructed human tissues to provide quantitative and robust information on local tolerance.
- Such data are predictive of human response, more accurate and discriminating compared to animal testing and, furthermore, they provide mechanistic information: being these models closer in term of morphology, barrier function, biochemical and physiological properties to *in vivo* human tissues, they represent today the most promising alternative to animals to investigate local tolerance and for fine tuning of toxicity mechanisms (early and delayed).

3Rs testing approaches for medicinal products

In accordance with Directive 2010/63/EU, the principle of the 3Rs (Replacement, Reduction and Refinement) needs to be considered when selecting testing approaches to be used for safety testing of human and veterinary medicinal products. In 2016 EMA issued a guideline to encourage stakeholders and authorities to initiate, support and accept the development and the use of 3Rs testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012). In particular, for non-clinical local tolerance testing of medicinal products, the use of validated and valid *in vitro* methods has been introduced as stand-alone tests or to be considered within a tiered testing strategy to eventually obviate the *in vivo* local tolerance test (EMA/CHMP/SWP/2145/2000 Rev. 1, Corr. 1*).

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