Trends in
Biotechnology

Review

Food allergen sensitization on a chip: the gut–immune–skin axis

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The global population is growing, rapidly increasing the demand for sustainable, novel, and safe food proteins with minimal risks of food allergy. *In vitro* testing of allergy-sensitizing capacity is predominantly based on 2D assays. However, these lack the 3D environment and crosstalk between the gut, skin, and immune cells essential for allergy prediction. Organ-on-a-chip (OoC) technologies are promising to study type 2 immune activation required for sensitization, initiated in the small intestine or skin, in interlinked systems. Increasing the mechanistic understanding and, moreover, finding new strategies to study interorgan communication is of importance to recapitulate food allergen sensitization *in vitro*. Here, we outline recently developed OoC platforms and discuss the features needed for reliable prediction of sensitizing allergenicity of proteins.

Highlights

Food allergen sensitization is characterized by a type 2 immune response towards a specific food protein and can adversely affect the patient after re-exposure of this food allergen.

The gut, skin, and lymph nodes (including immune cells) are interconnected key organs in food allergen sensitization.

Gut and skin organ-on-a-chip (OoC) devices have been developed with an immune component to recapitulate the 3D epithelial cell–immune cell crosstalk, although specialized gut–immune–skin OoC models to study food protein sensitizing allergenicity capacity are not available yet.

The inclusion of compartmentalized innate and adaptive immune cells is crucial to mimic the immune cascade in the gut–immune–skin axis in a stepwise manner.

Engineering a gut–immune–skin axis OoC can better evaluate food allergen sensitization in the future and advance mechanistic insight.

The rising prevalence of food allergies and the need for predictive *in vitro* models

Over the past couple of decades, allergies have been increasingly recognized as an emerging public health concern. Food allergy is commonly diagnosed as an IgE-mediated allergy against a food protein, with rapid clinical onset of local and/or systemic symptoms including anaphylaxis. A prevalence of up to 10% has been reported for children of both westernized and developing countries [1,2]. It has been widely accepted that particularly infants and children are affected. Children can outgrow their allergy and, depending on the allergen, also the risk of persistent food allergy differs. However, it should be realized that food allergy in early life is recognized as a key risk factor for development of secondary allergies, including type 2 asthma later in life. In general, up to 6.5% of adults have clinically diagnosed food allergies and recent studies indicate also a rise in the onset [2]. This poses a burden on individual patients accompanied by substantial health care costs [1–3]. The acute immunological hypersensitivity reaction occurs upon food protein induced IgE crosslinking of Fcε receptors on allergic effector cells. Type 2 immune responses against a specific food protein, referred to as the **food allergen** (see Glossary), lead to the secretion of allergen-specific IgE in a process known as allergic sensitization. Clinical manifestations of acute IgE-mediated allergic responses involve gastrointestinal, systemic cutaneous, and respiratory complications and may even lead to anaphylaxis. Foods causing acute allergic symptoms most commonly include hen's egg, cow's milk, peanuts, soybeans, tree nuts, crustacea, wheat, soy, fish, and shellfish [4]. In contrast to IgE-mediated food allergies (>90% of cases), non-IgE-mediated food allergies (<10% of cases) provoke chronic and delayed inflammation mainly localized in the intestine due to heterogenous cellular responses resulting in food protein induced enteropathy or colitis syndrome [4,5]. This review focuses on the onset of IgE-mediated food allergies.

Food allergy is composed of two phases in which sensitization to a specific food allergen is followed by an elicitation phase which manifests in allergic symptoms when re-exposed to the same allergen. However, other than avoidance of food allergens and epinephrine injections in severe cases after accidental exposure, successful treatments against food allergy are not available. For years, it has been

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assumed that gastrointestinal allergen exposure was the sole route responsible for the development of food allergies. However, there is substantial evidence for the role of the skin in food sensitization as well (Box 1 and Figure 1), highlighting a connection between the gut, skin, and immune system (Box 2). Representative *in vitro* models to predict the immune sensitizing capacity of newly introduced food allergens in our diets are currently lacking. Since it has been shown that multi-**organ-on-a-chip (OoC)** systems more closely recapitulate complex *in vivo* interorgan communication, these systems offer promising tools for assessment of **food allergen sensitization** as this would allow for combining the gut, skin, and immune system in one single model. This review addresses the current understanding of food allergen sensitization in the gut-immune–skin axis and how this mechanism can be assessed in OoC models.

3D gut–immune and skin–immune *in vitro* models

Immunocompetent 3D *in vitro* intestinal models

2D *in vitro* intestinal epithelial cell (IEC) models are well developed, and although IECs can be cocultured with immune cells, integration of a compartmentalized immune system is complex and still lacking. Recently, an advanced 2D *in vitro* model, combining dendritic cells (DCs), T cells, and B cells in a multiple step sequential coculture assay to mimic food allergen sensitization via IEC exposure to ovalbumin (OVA) [6] and, extended with mast cells [7], proved already a valuable step closer to the desired gut physiology and complexity. However, immune cell migration and direct IEC–immune cell crosstalk is not covered in this model as cells were kept solely in medium. Advances have been made to increase complexity under physiologically relevant conditions using **3D** *in vitro* IEC models. For example, intestinal villi-like 3D models were generated with intestinal organoids seeded onto prepatterned hydrogels encapsulating intestinal fibroblasts [8], while other cells, for example, immune cells, could in the future be encapsulated in these models.

Immunocompetent 3D *in vitro* skin models

Advancements in skin equivalents have been made to develop immunocompetent models. Langerhans cells (LCs) are skin-resident cells populating the epidermis under physiological conditions. Coseeding LCs that were differentiated from the human $CD34^+$ DC precursor cell line MUTZ-3, together with keratinocytes, has been widely used for a fully stratified epidermis comparable with native human skin [9–13]. Additionally, upon allergen or irritant exposures, MUTZ-3-LCs migrate to the dermis [9,11,12] and upon attraction of several epidermal-derived chemokines, cells were able to migrate through the dermis thereby repopulating the epidermis [13]. Similar to LCs, monocyte-derived DCs have been coseeded together with human skin

Box 1. The role of the skin in food sensitization

Being the largest organ in the human body and the first to encounter environmental threats [64], the skin has emerged as a potential site of food allergen sensitization (see Figure 1 in the main text) [4,65]. In the skin, initiation of sensitization occurs when epidermal-resident LCs or dermal DCs capture a particular food allergen, which would have entered the dermis most commonly by penetration through a damaged barrier [13]; however, entrance through an intact barrier has also been observed [66]. In rodents, epicutaneous exposure to hen's egg allergen OVA followed by an oral OVA challenge induced IgE-mediated food allergic symptoms [66–68]. Allergic sensitization via the skin was also found for epicutaneous exposure to gluten [69], α-lactalbumin [70], and peanut oil [71], which all resulted in rise in allergen-specific IgE levels. In these studies tape stripping, damaging the skin, was applied before allergen exposure or intact skin was used. In humans, it is well known that infants with AD, a condition characterized by an impaired epidermal barrier, are at higher risk of developing food allergies [72,73]. In AD patients, the skin permeability is enhanced, facilitating allergen penetrance, but also the AD-related epidermal inflammation contributes to increased risk for food sensitization [74]. Furthermore, proteins used in body creams and/or soaps have been associated with food allergy development. For example, epidemiological studies involving Japanese women showed a correlation between the use of hydrolyzed wheat protein-containing soap and wheat allergy [75,76]. These findings introduce the skin as an additional route for food allergen sensitization (see Figure 1 in main text). However, the complete skin–gut sensitization route is yet not fully understood (see Box 2 in the main text). Advanced, physiologically relevant gut–immune–skin axis *in vitro* models can help to further elucidate the immunological mechanisms of food allergen sensitization in both the gut and skin.

Glossary

2D: 2D *in vitro* assays are referred to as cell culture on a flat surface, such as used in standard cell culture wells plates or flasks.

3D: 3D *in vitro* assays include cell growth in multiple directions, forming 3D-like structures, often in combination with ECM embedding (e.g., collagen) or cell-compatible scaffolds.

Chemokine ligand: (or chemokines), are proteins that are involved in the directional migration of immune cells, based on forming a chemoattractant gradient, for example a C-C chemokine ligand (CCL)20 gradient secreted by the epithelium to attract immune cells such as DCs.

Chemokine receptor: expressed by, among others, immune cells to be able to migrate towards a chemoattractant gradient into a specific tissue, using their specific chemokine receptors to selectively bind to specific CCL.

Food allergen: food particles that are responsible for the induction of a food allergy.

Food allergen sensitization: induced by an interplay between a specific food protein (allergen) and the host's immune system, which in most cases is an IgE-mediated response leading to a food allergen-specific IgE response by B-cell-derived plasma cells.

Microfluidics: devices characterized by microscaled small fluid-containing channels to, for example, apply shear stress and supply nutrients to cells or to connect different cell types.

Organ-on-a-chip (OoC): advanced *in vitro* 3D models that physiologically and mechanically can simulate specific characteristics of a specific organ, often in combination with microfluidic channels.

Polydimethylsiloxane (PDMS) device: is a frequently used polymer to reconstitute OoC and microfluidic devices.

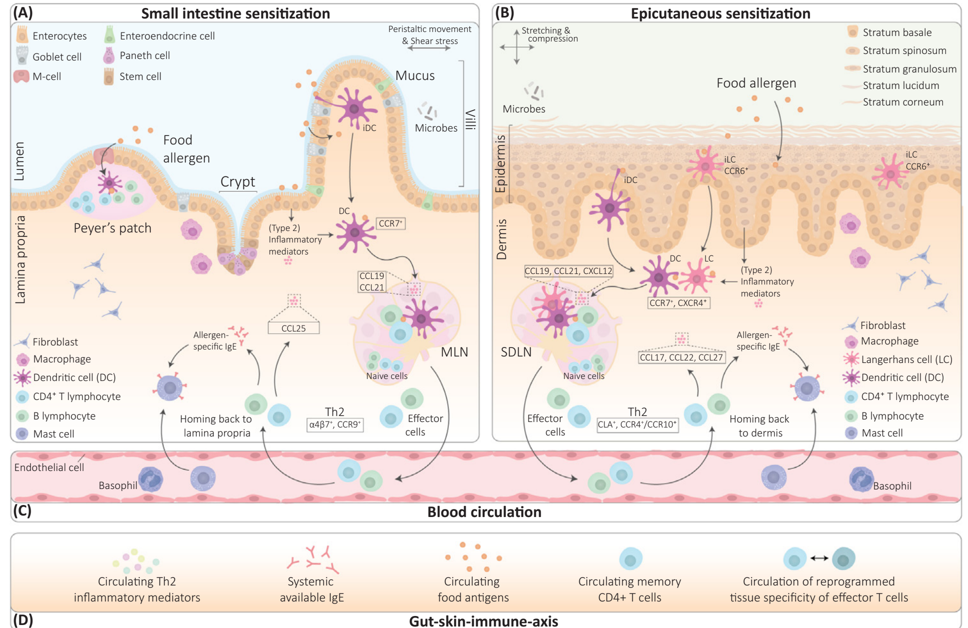

Figure 1. Overview of the gut–immune–skin axis in food allergen sensitization. Food allergen sensitization at the intestinal (A) and epicutaneous (B) site are recognized by comparable molecular initiating and subsequent key events [36,37]. Dendritic cells (DCs) in the intestine or DCs and Langerhans cells (LCs) in the skin, capture penetrated food allergens and are directed towards a type 2 phenotype by epithelial secreted type 2 inflammatory mediators [37–43]. These intestinal DCs travel to the mesenteric lymph nodes (MLNs) [37] and epicutaneous DCs and LCs travel to the skin draining lymph nodes (SDLNs) [42,44–49]. Naïve T cells in the MLNs and SDLNs can then be directed towards a T helper (Th)2 phenotype and express site-specific homing markers, for example, under the influence of retinoic acid (gut) or vitamin D3 (skin) [36,37,42,45]. Subsequently, B cells in the MLNs and SDLNs bind soluble allergen and are instructed towards allergen-specific IgE-producing plasma cells. These activated T and B cells enter the circulation (C) and home back to the lamina propria in the intestine or the dermis in the skin [54–58]. The gut–immune–skin axis (D) has been proposed to involve circulating Th2 inflammatory mediators and systemically available IgE [77,78], circulating food antigens [79–81], circulating memory CD4^{+} T cells [57,82], and circulation of reprogrammed tissue specificity of effector T cells [58,83,84]. Abbreviations: CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; CXCR, C-X-C chemokine receptor.

equivalents and were localized in the epidermis and dermis, respectively [14]. A hydrogel containing encapsulated DCs incorporated in between keratinocytes and fibroblasts, allowed for analyzing individual skin equivalent layers while maintaining the capability of bidirectional migration of DCs [15]. Additionally, the incorporation of T cells into skin equivalents allowed studying their interactions with the epidermis [16,17].

Currently available 3D gut and skin models are still limited as these models lack complexity because of the absence of, for example, vascularization, innervation, and more complex immune cell–immune cell crosstalk, as well as essential physiological mechanical properties, compromising the dynamic nature of the organs. For the purpose of allergen sensitization, mobilization of the DCs or LCs via afferent lymphatics to the gut mesenteric lymph nodes (MLNs) or skin draining lymph nodes (SDLNs) should be modeled, since here naïve T and B cells are instructed to develop into type 2 effector cells.

Current state-of-the-art gut–immune OoC and skin–immune OoC *in vitro* models

For decades, animal models and *in vitro* studies have been used to study intestinal diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and food allergy, as well as epicutaneous diseases such as atopic dermatitis (AD), psoriasis, or irritant exposure. However,

Box 2. Routes in the gut–immune–skin axis in food sensitization

Circulating Th2 inflammatory mediators and systemic IgE

Food allergens can activate skin or gut epithelium resulting in the release of interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP), which are considered key communicators between the skin and gut. Increased circulating levels of IL-33 and TSLP in AD patients can activate LCs, type 2 innate lymphoid cells (ILC2s), and Th2 cells [77]. Circulating Th2 mediators could potentially lead to mast cell precursor migration towards the intestinal tissue [77] and, with regard to food sensitization (either via the skin or gut), possibly be opsonized by circulating allergen-specific IgE. Enhanced IL-33 serum levels in mice were associated with activation of IL-13- and IL-4-secreting intestinal ILC2s, which could also increase epithelial permeability and potentially drive increased expansion and activation of mast cells [78].

Circulating food antigens

Following the absorption–deposition hypothesis [79], intact food allergens could become systemically available and deposited to distant sites, provoking a proallergy response in other organs. Ara h 6 [80], or OVA and β-lactoglobulin [81], have been detected in systemic circulation, in particular when barrier function was diminished.

Circulating memory CD4$^+$ T cells

T cell homing towards the affected tissue is instructed by tissue-specific DCs in the tissue-specific draining LNs. Homing receptors for skin are dependent on environmental cues, such as the active metabolite of vitamin A, retinoic acid, in the gut or the active metabolite of vitamin D3, 1,25(OH)$_2$D$_3$, in the skin. Both vitamins can be processed to their active form by tissue-specific antigen presenting DCs [57]. Circulating food-allergen-specific CD4$^+$ T cells were found in AD and food allergic children [82]. These studies highlight circulating memory CD4+ T cells, which might suggest a, yet unexplored, role at different sites of the body.

Reprogramming of tissue specificity of effector T cells

In mice, it was found that adoptively transferred CD4$^+$ α4β7$^+$ effector T cells from the intestinal MLNs, can migrate to the skin only when expressing CCR4, possibly by reprogramming them in the MLNs to home to the antigen-challenged skin. This mechanism could explain skin flares, seen in orally sensitized AD patients, after epicutaneous contact with food allergens [58]. Although effector T cells often contain tissue-specific homing receptors, studies have shown that these tissue-tropic T cells can be reprogrammed by tissue-specific DCs to change organ specificity of these tissue effector T cells to either the intestine or skin and vice versa [83,84].

animal models have societal and ethical considerations and their translational value towards humans can be inaccurate. Less complex 2D and 3D *in vitro* models do not have these ethical restrains; however, they often lack physiological complexity and interorgan crosstalk. Therefore, development of OoC platforms in which multiple organs can be combined to study interorgan crosstalk in real time and under continuous flow, could have added value to reduce or replace the use of *in vivo* models.

Immunocompetent gut OoC models

Several attempts have been made to include immune cells in a gut OoC system, and, in the scope of this review, we only include gut–immune OoC platforms published between 2019 and 2023 (Table 1 and Figure 2). As allergy OoC systems currently do not exist, we focused on systems available to study gut inflammation or viral infection. These include multichannel **microfluidic** chips with dynamic (e.g., peristaltic movement and flow) and functional elements of the intestine [18,19] and the multilane OrganoPlate from MIMETAS, with gravitational flow [20–22] (Figure 2A). The latter has the unique design of three channels aligned without physical barriers, thereby promoting cell–cell interactions. An inflammatory gut model was designed by separating an intestinal outer channel, a 3D extracellular matrix (ECM) middle channel, and an outer immune channel including macrophage-like (THP-1) and DC-like (MUTZ-3) cells [20]. Similarly, but with human, patient-derived primary intestinal organoids and monocyte-derived macrophages, TPCA-1 could prevent the inflammatory response in a dose-dependent manner [21]. Furthermore, extending this MIMETAS model with THP-1 cells incorporated in the middle ECM channel and circulating freshly isolated neutrophils in an outer channel

Table 1. Detailed overview of recently developed immunocompetent skin OoC and immunocompetent gut OoC models[a]

Model design	Fabrication technique	Cells	Advantages	Challenges	Refs
Immunocompetent Gut OoC models					
OrganoPlate with three channels per culture chip within the plate. Caco-2 and HT29-MTX-E12 mixture seeded in the upper medium channel, lined by the ECM middle channel and a mixture of macrophage-like (THP-1) and DC-like (MUTZ-3) cells in the lower channel.	3-Lane OrganoPlate (MIMETAS)	Caco-2, HT29-MTX-E12, THP-1, MUTZ-3	- Commercially available: optimized and controlled system of OrganoPlate - Pumpless and controlled flow - High throughput - Absence of physical barrier between cells - Measurement of barrier integrity by TEER - Easy access to apical and basolateral side - Model intestinal inflammatory processes	- Products need to be purchased that are compatible with OrganoPlate - Design of device not tunable - Limited application of immune cells; only macrophage-like and DC-like cell lines included in the model - Limited immune cell characterization	[20]
A three-lane OrganoPlate system, integrated with human intestinal organoids at one of the side channels, followed by an ECM separation hydrogel embedded with primary macrophages, which lines the other outer channel containing medium.	3-Lane OrganoPlate (MIMETAS)	Human intestinal organoids, monocyte-derived macrophages	- Commercially available: optimized and controlled system of OrganoPlate - Pumpless and controlled flow - High throughput - Absence of physical barrier between cells - Easy access to apical and basolateral side - Model intestinal inflammatory processes	- Products need to be purchased that are compatible with OrganoPlate - Design of device not tunable - Limited application of immune cells: only monocyte-derived macrophages included - Limited immune cell characterization	[21]
Multichamber microfluidic system with an intestinal tube on one site, lined with ECM embedded macrophage-like cells in the middle channel and circulating neutrophils on the other outer channel.	3-Lane OrganoPlate (MIMETAS)	Caco-2, THP-1, Neutrophils (freshly isolated)	- Commercially available: optimized and controlled system of OrganoPlate - Pumpless and controlled flow - High throughput - Absence of physical barrier between cells - Easy access to apical and basolateral side - Simulated interactions between epithelial barrier and ECM-residing macrophage-like cells and migratory neutrophils - Active immune cell migration and neutrophil invasion	- Products need to be purchased that are compatible with OrganoPlate - Design of device not tunable - Limited application of immune cells: Only includes macrophage-like cells and neutrophils	[22]
An ECM-coated two-channeled device which included seeding of intestinal organoids in the apical compartment and HIMECs in the basolateral compartment. PBMCs were subsequently included in the basolateral endothelial channel (adherent and circulating).	Intestine chip; S-1 Chips (Emulate Inc)	Human intestinal organoids (patient duodenal), HIMECs, PBMCs	- Commercially available: optimized and controlled system - Controlled perfusion and cyclic, peristaltic and mechanical deformations - Endothelial cells are exposed to adherent and circulating PBMCs - Study coronavirus infection/inflammatory responses in the intestine - Vasculature incorporation	- Products need to be purchased that are compatible with Emulate's chip - Design of device not tunable - No specific immune cell subset/response studied: cocktail of different immune cells - Limited immune cell characterization	[23]
A two-channeled device with endothelial cells and IECs, including immune cells and lactobacilli. HUVECs were inserted in the upper chamber, with seeded macrophages on top. Caco-2 cells were seeded in bottom chamber. Endothelial chamber was finally perfused with PBMCs.	MOTiF biochips (microfluidic ChipShop GmbH), with an integrated PET membrane	HUVECs, Caco-2, PBMCs, primary macrophages	- Commercially available; customized designs possible - Controlled perfusion - Coculture of microbial strains - Tissue-resident innate immune cells: with mucosal macrophage and DC features - Perfusion with PBMCs to mimic infiltration by leukocytes during inflammation of the intestine - Vasculature incorporation	- Limited to options of chip fabrication	[24]

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Table 1. (continued)

Model design	Fabrication technique	Cells	Advantages	Challenges	Refs
A two-layered device, including two channels, which are separated by a porous ECM-coated PDMS membrane. Vascular endothelial cells were first seeded on the membrane bottom side, followed by seeding of a mixture of Caco-2 and HT-29 cells in the upper channel. In addition, lower channel contains circulating PBMCs.	Soft lithography, material final device: PDMS, including a porous PDMS membrane	Caco-2, HT-29, HUVECs, PBMCs	- Tunable design - Controlled perfusion - Study of viral infection, transmission, and host–virus interactions. - Resembles intestine- and endothelial specific responses towards SARS-CoV-2 - Vasculature incorporation	- Material is permeable for small substances/molecules - Mainly epithelium and endothelium were characterized - No specific immune cell subset/response studied: cocktail of different immune cells - Limited immune cell characterization	[25]
Three-layered and three channeled microfluidic chip. Each channel is separated with an ECM-coated porous PDMS membrane that enables multicell co-culture. In the middle channel, Caco-2 cells were seeded and HUVECs were seeded on the basal side of both separation membranes. Macrophages were then introduced in the vascular lumen.	Soft lithography, material final device: PDMS, including two porous PDMS membranes	Caco-2, HUVECs, U-937	- Tunable design - Peristaltic movement - Controlled perfusion - Mimicking of intestinal inflammation through application of the combination of *Escherichia coli* and *Lactobacillus casei* and macrophages. - Allows for host–microbial interaction study in a microsystem - Vasculature incorporation	- Material is permeable for small substances/molecules - Limited application of immune cells: only macrophages derived from monocyte cell line were included - Limited immune cell characterization	[26]
A two-layered PDMS device separated by an ECM-coated porous PDMS membrane. Caco-2 cells lined on one side of the membrane and HUVECs were cultured at the basal side of the membrane. *E. coli* was introduced in the epithelial channel as well as macrophages in the endothelial channel.	Soft lithography, material final device: PDMS, including a porous PDMS membrane	Caco-2, HUVECs, Primary human macrophage cells	- Tunable design - Peristaltic movement - Controlled perfusion - Study properties of COS and possibly other immunomodulatory substances - Co- - culture of microbes - Vasculature incorporation	- Material is permeable for small substances/molecules - Limited application of immune cells: only macrophages were included - Limited immune cell characterization	[27]
Two-layered PDMS construct with a PC porous membrane in between channels and cell chambers, which formed the gut-on-a-chip microfluidic device. Lower chamber included intestinal cells, endothelial cells in the upper chamber (formed the capillary site) and upper chamber perfused with PBMCs.	PDMS layers casted on silicon wafers, material final device: PDMS, including a PC porous separation membrane	Caco-2, HUVECs, PBMCs	- Tunable design - Controlled perfusion - Perfused Caco-2 layer (multi-channel injection pump) - Vasculature included - Inflammatory bowel disease model, including microbes - Model is applicable to mimic different diseases in the intestine	- Material is permeable for small substances/molecules - No specific immune cell subset/response studied: cocktail of different immune cells - Limited immune cell characterization	[28]
Immunocompetent Skin OoC models					
Chip with three culture chambers, two chambers are characterized by a 24 well format (to co-culture RHG and RHS-LC) and one chamber a 96 well format (for medium exchange)	HUMIMIC Chip3plus (TissUse GmbH)	Human primary keratinocytes and fibroblasts (skin and gingiva from surgical waste), MUTZ-3	- Commercially available: optimized and controlled system - Controlled pulsatile fluid flow - Study systemic toxicity of for example metals - Inclusion of tissue-resident LCs - Human primary RHG and RHS-LC (fibroblasts plus keratinocytes) - LC migration	- Backbone design of device not tunable - Limited application of immune cells: Only MUTZ-3 derived LCs were included	[29]

Table 1. (continued)

Model design	Fabrication technique	Cells	Advantages	Challenges	Refs
A three-channeled device with on one side dermal fibroblasts, in the middle a 3D ECM-containing HUVECs and the other lateral channel containing macrophages.	Soft lithography, material final device: PDMS	Human dermal fibroblasts, HUVECs, Monocyte-derived M1 and M2 macrophages (differentiated from freshly isolated monocytes)	- Tunable design - Vascularization in 3D ECM - Both M1 and M2 macrophages studied - Simulates inflammatory environment	- Material is permeable for small substances/molecules - Lack of keratinocytes - Lack of fully differentiated skin equivalents - No tissue-resident LCs - Limited application of immune cells: only monocyte-derived M1 and M2 macrophages - Limited immune cell characterization	[30]
Two-layered PDMS device separated by a porous membrane. The upper part included multiple chambers, including for the skin equivalent and for cell culture medium. The lower layer contained the fluidic channel together with the endothelial cells underneath the porous membrane and HL-60 cells in circulation.	Soft lithography, material final device: PDMS, including a porous membrane	Human dermal primary fibroblasts, HaCaT or primary keratinocytes, HUVECs, HL-60 (differentiated into neutrophil-like cells)	- Tunable design - Passive perfusion (based on rocking platform) - Dermal and epidermal part in skin equivalent - Vasculature incorporation - Neutrophil infiltration in skin tissue/transmigration	- Material is permeable for small substances/molecules - No tissue-resident LCs - Limited application of immune cells: only neutrophil-like cells were included - Limited immune cell characterization	[31]
Skin-on-a-chip model with three compartments, one lined with HUVECs, the middle to allow for immune cell migration through a 3D collagen gel, and the third compartment with HaCaT cells.	Photolithography, material final device: PDMS	HaCaT, HUVEC, human peripheral blood T cells, human blood neutrophils	- Tunable design - Controlled chemical gradient application - Vasculature incorporation - Clear and easy tracking of immune cell migration - Includes trans-endothelial and -epithelial immune cell migration	- Material is permeable for small substances/molecules - Lack of dermal fibroblasts - Lack of fully differentiated skin equivalents - No tissue-resident LCs - Limited application of immune cells: Only T cells or neutrophils were included - Limited immune cell characterization	[32]
Device with a microvascular network constructed by injection molding. Vascular network is enclosed within collagen constructs including fibroblasts, which was stationed between plexiglasses. The upper plexiglass has two injection inlets for collagen, one inlet and one outlet to perfuse endothelial cells and cell culture medium and one air-liquid interphase well for keratinocytes.	Injection molding, photolithography and soft lithography, material final device: plexiglass	Human primary dermal fibroblasts, Human primary epidermal keratinocytes, Human primary dermal microvascular endothelial cells, Neutrophils (isolated from whole blood)	- Tunable device - Controlled perfusion - Dermal and epidermal part in skin equivalent - Vasculature incorporation - Studying host-immune responses towards viral infections - Neutrophilic trans-endothelial extravasation - Directional migration of neutrophils	- No tissue-resident LCs - Limited application for immune cells; only neutrophils included	[33]

[a]Abbreviations: HIMECs, human large intestine microvascular endothelial cells; PET, polyethylene terephthalate; RHG, reconstructed human gingiva; RHS-LC, reconstructed human skin with MUTZ-3-derived Langerhans cells (MUTZ-3-LC); TEER, transepithelial electrical resistance.

allowed study of neutrophilic infiltration across the cellular barrier, thereby more closely mimicking intestinal inflammation [22]. Another commercially available device, from Emulate, was used to study coronavirus infection in a multichannel Emulate S-1 Chip. Human intestinal organoids were included in one channel and endothelial cells lined the other channel combined with circulating peripheral blood mononuclear cells (PBMCs) and were exposed to continuous flow and cyclic mechanical deformations (Figure 2B). By including vasculature and mechanical stresses, this device added relevant

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Figure 2. Overview of recently developed immunocompetent gut organ-on-a-chip (OoC) and skin OoC platforms. Immunocompetent gut OoCs (A–F) and immunocompetent skin OoCs (G–K) platforms were developed between 2019 and 2023. (A) 3-Lane OrganoPlate (MIMETAS) with human intestinal organoids and monocyte-derived macrophages [21]. (B) S-1 Chips (Emulate Inc.) with cocultured human intestinal organoids, human large intestine microvascular endothelial cells (HIMECs), and peripheral blood mononuclear cells (PBMCs) [23]. (C) MOTiF biochips (microfluidic ChipShop GmbH) with cocultured Caco-2 cells, human umbilical vein endothelial cells (HUVECs), PBMCs, and primary macrophages [24]. (D)

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mechanical complexity to the gut–immune OoC [23]. In addition, the commercially available MOTiF biochips with an integrated polyethylene terephthalate (PET) membrane were used to create an endothelial channel with resident innate immune cells and circulating PBMCs, combined with an IEC channel including microbes. This model resembled more closely mucosal immunity (Figure 2C) [24].

Several other studies have aimed for the coculture of IECs with endothelial cells in in-house **polydimethylsiloxane (PDMS) devices** separated by using porous PDMS separation membranes [25–28]. Such application was used to study pathophysiological features of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected intestinal epithelial barrier by culturing IECs in one channel and endothelial cells including circulating PBMCs in the other channel. This led to disrupted barrier integrity, however, the immune response itself was not addressed, but solely the endothelial cell inflammatory effects as well as IECs (Figure 2D) [25]. Furthermore, devices were developed with cocultures of either intestinal, endothelial, and macrophage cell lines [26] or primary human macrophages [27], under peristaltic movement. Both models, in combination with intestinal microbes, showed *Escherichia-coli*-provoked epithelial damage and an intestinal inflammatory response. *Lactobacillus casei* and antibiotics reduced epithelial damage and decreased inflammatory responses (Figure 2E) [26], and similar immunomodulatory effects of chitosan oligosaccharides (COSs) on enteritis were observed (Figure 2F) [27]. In line with these PDMS casted devices, IECs and endothelial cell lines were cocultured separated by a polycarbonate (PC) membrane, allowing for host–microorganism interaction with circulating PBMCs and patient-derived microbes to study enteritis and the effect of probiotics [28].

Immunocompetent skin OoC models

For skin OoC technology as a screening tool for food allergen sensitizing allergenicity risk, incorporation of immune cells into the dynamic system should be acquired to model relevant cell types involved in sensitization via the skin, similar to the gut OoC. In the scope of this review, we included recently published skin–immune OoC platforms only (Table 1 and Figure 2).

The skin–immune OoC has been developed and improved too, although, as of yet, not to the level of immunocompetent gut OoC platforms. And similar to gut OoC, food allergy sensitization models are not available. A commercially available device, HUMIMIC Chip3plus (TissUse), was used to culture reconstructed human gingiva (RHG) as well as reconstructed human skin (RHS) with MUTZ-3-LC cells (RHS-LC). This model proved to activate and mature LCs of the RHS, and demonstrated migration of LCs into the dermis upon exposure of RHG to NiSO4 (Figure 2G) [29]. Although just one immune cell type was included, this model covers tissue-resident LCs in a full skin equivalent and migration of these LCs from the epidermis into the dermis.

Polydimethylsiloxane (PDMS) device with cocultured colon carcinoma (Caco-2) cells, human Caucasian colon adenocarcinoma (HT-29) cells, HUVECs, and PBMCs [25]. I PDMS device with cocultured Caco-2 cells, HUVECs, and U-937 cells [26]. (F) PDMS device with cocultured Caco-2 cells, HUVECs, and primary human macrophages [27]. (G) HUMIMIC Chip3plus (TissUse GmbH) with cocultured human primary keratinocytes, human primary fibroblasts, and Menschliche Und Tierische Zellkulture-3 Langerhans cells (MUTZ-3-LC) [29]. (H) PDMS device with cocultured human dermal fibroblasts, HUVECs, and M1 and M2 macrophages (differentiated from freshly isolated monocytes) [30]. (I) PDMS device with cocultured human dermal primary fibroblasts, HaCaT or primary keratinocytes, HUVECs, and HL-60 cells [31]. (J) PDMS device with cocultured immortal adult human skin (HaCaT) cells, HUVECs, human T cells, and human neutrophils [32]. (K) Device of plexiglass including collagen with cocultured human primary dermal fibroblasts, human primary epidermal keratinocytes, human primary dermal microvascular endothelial cells, and neutrophils (isolated from whole blood) [33]. Images were reused or adapted, with permission, from the references cited in the legend. Panels A, B, E, F, and K adapted under a Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). Panel G (Image owner is TissUse GmbH) adapted under a Creative Commons CC BY-ND 4.0 license (https://creativecommons.org/licenses/by-nd/4.0/). Abbreviations: EC, endothelial cell; ECM, extracellular matrix; IEC, intestinal epithelial cell; mMphs, mucosal phagocytic macrophages; RHG, reconstructed human gingiva; RHS-LC, reconstructed human skin with MUTZ-3-LC cells; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

A three-channeled PDMS device with dermal fibroblasts on one side, 3D ECM containing an endothelial cell line in the middle, and monocyte-derived M1 or M2 macrophages on the other side is available to study wound healing. Nevertheless, keratinocytes were not included (Figure 2H) [30]. Leukocyte migration towards the site of skin inflammation was studied by using a pumpless microfluidic system, composed of either immortalized human keratinocyte HaCaT cells or primary keratinocytes and human primary fibroblasts, forming a skin equivalent on one side of the permeable PDMS membrane and human umbilical vascular endothelial cells (HUVECs) on the other. In case the endothelial compartment was perfused with a suspension containing a neutrophil-like cell line, following UV irradiation and chemical exposure, the cells successfully migrated across the endothelial layer into the dermis (Figure 2I) [31]. T cells have also been successfully incorporated in a multichannel PDMS microfluidic skin OoC device in which T cell migration in response to skin inflammation, induced by chemokine gradients of C-X-C **chemokine ligand (**CXCL)12 and C-C chemokine ligand (CCL)20, was evaluated. Even though the migratory capacity of T cells from the HUVEC channel towards HaCaT cells through a 3D collagen hydrogel has been established and was inhibited by a CCL20 locked dimer, further research should examine the functionality of these T cells in the recreated 3D environment (Figure 2J) [32].

Recently, a device with a more advanced vasculature was developed, composed of human primary dermal microvascular endothelial cells lining multiple microchannels embedded in human primary dermal fibroblasts. Human primary keratinocytes were cultured on top to recreate a full skin equivalent and freshly isolated neutrophils circulated in the microvessels. With this immunocompetent skin OoC device, an epicutaneous herpes simplex virus infection and ulceration of the skin could be modeled, which was applied to study an antiviral drug (Figure 2K) [33].

Challenges in current immunocompetent gut and skin OoC platforms

Most of the highlighted immunocompetent skin OoC platforms include directional migration of predominantly innate immune cells, while most of immunocompetent gut OoC systems fail to facilitate this. Alternatively, PBMCs are often added to immunocompetent OoC platforms; however, immune compartmentalization of the gut or skin is not taken into account. Immune cell migration or transmigration of innate or adaptive immune cells is not only relevant for changing location or for migration from or towards (draining) LNs or tissues, but also for intercellular crosstalk by antigen-presenting cells (APCs) such as DCs and skin LCs, leukocytes, and tissue-specific cells [34]. The reviewed gut–immune and skin–immune OoC systems (Table 1) mostly include only one or two, mainly innate, immune cell subsets, without recapitulating the complete immune cascades. For the development of sensitizing allergenicity risk OoC platforms, multiple immune cells should be included, such as DCs, T cells, B cells, and mast cells in separated compartments to apply gut–skin OoC technology.

Immunocompetent gut–skin axis OoC

To the best of our knowledge, gut–immune–skin axis OoC models have, as of yet, not been developed, highlighting a valuable direction for future research. One recent study combined both the gut and skin within one device, resulting in a coculture of IECs, dermal fibroblasts, and keratinocytes. The two epithelial sites are connected via a basolateral medium channel. Although immune cells were not included, the study demonstrated how intestinal inflammation can affect the skin by showing the elevation of the psoriasis-associated marker hBD-2 in the skin [35].

The need for immunological compartmentalization in food allergen sensitization OoC models

The inclusion of molecular initiating events (MIEe) followed by key events (KEs) is crucial [36,37] for future physiologically relevant gut–immune–skin axis OoC devices (Figure 1 and Figure 3, Key figure).

Key figure

Overview of a compartmentalized gut–immune–skin organ-on-a-chip (OoC) platform

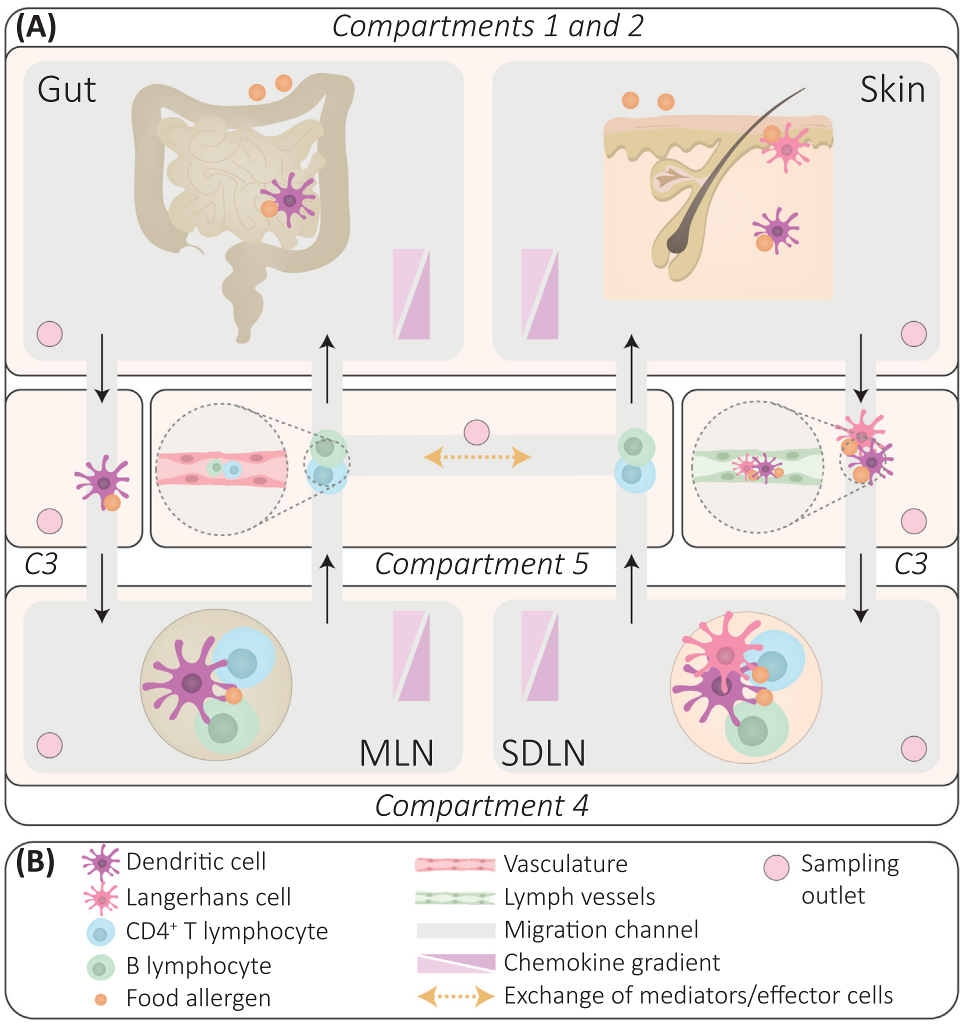

Figure 3. (A) To develop a compartmentalized and functional gut–immune–skin OoC device, the gut or skin could be exposed to food allergens in the first compartment, and cocultured with antigen-presenting cells (APCs) in the second compartment. These two compartments are then linked to the mesenteric lymph nodes (MLNs) (gut) or the skin draining lymph nodes (SDLNs) (skin) via a lymph migration channel (compartment 3; C3) from the site of the lamina propria in the gut or dermis in the skin respectively. In compartment 4, naïve T cells may be activated by migrated food-allergen-presenting APCs via the T cell receptor selective for the allergen, while B cells may recognize the allergen via the B cell receptor, and present it via MHC-II to these activated interleukin (IL)-4-producing allergen-specific T cells. This results in IgE-producing effector B cells/ plasma cells also with further help from follicular T helper cells. Once activated, effector T and B cells can home back to the lamina propria in the gut or the dermis in the skin via migration channels representing the vasculature, highlighting compartment 5. Furthermore, to connect the circulation of both skin and gut, allowing for cell migration and mediator exchange, an additional migration channel is positioned between the two vasculature channels in compartment 5. In every compartment, sampling outlets are included to allow for real time measurements. (B) Legend for A.

Compartments 1 and 2: the epithelial barrier and its tissue-specific ECM

The OoC platform should include a first compartment where food allergens can activate and/or translocate over IECs or epicutaneous epithelial cells. Once allergens have crossed the epithelial barrier, they need to be taken up by intestinal DCs in the lamina propria or Peyer's patches of the gut, or epicutaneous LCs and DCs in the epidermis or the dermis, respectively. This highlights the second compartment of the OoC platform separately for both the skin and intestine: skewing the DCs and LCs towards a type 2 phenotype, in the presence of type 2 inflammatory mediators excreted from the epithelium [37–43]. In the intestine as well as the skin, CCL20 attracts C-C **chemokine receptor** (CCR)6-expressing immature DCs [39,40] and, in the case of the skin, also LCs [39]. Both type 2 skewed intestinal DCs as well as type 2 skewed epicutaneous DCs and LCs express activation markers and differentiation markers [37,42,44,45].

Compartment 3: APC homing towards LNs

For correct LN homing, via compartment 3, intestinal DCs mainly upregulate CCR7 when sensitized [37], ligating with CCL19/CCL21 in the MLNs, while skin DCs and LCs upregulate C-X-C chemokine receptor (CXCR) 4 and CCR7 that ligate with CXCL12 and CCL19/CCL21 respectively [46–49]. Migration channels can vary in size and differ in fabrication method used to create such channels. Although different 3D printing technologies are available, one should mainly focus on channel length to not hamper chemokine diffusion, as well as channel width, to allow for easy immune cell migration. More specifically focusing on the origin of migration channels, hydrogels with migratable pore sizes as well as functional sites for adhesion of migrating cells could be a first step towards such a channel. Hydrogels can vary with origin; however, collagen-I-based hydrogels have most extensively been used while studying immune migration [50,51]. Besides protein-based hydrogels, biomimetic synthetic hydrogels have also been used to study immune cell migration [52]. To further increase complexity, migration channels could be designed to allow for lymphatic endothelial cell culture inside the channel, and subsequently to apply flow.

Compartment 4: antigen presentation and T and B cell activation in the LNs

In compartment 4 of the OoC platform, the LN compartment, naïve T cells can be introduced which can be activated by type 2 DCs (or LCs) since these produce type 2 polarizing proinflammatory cytokines and express co-stimulatory molecules. Subsequently, naïve T cells in the MLNs and SDLNs can be directed towards a T helper (Th)2 phenotype [36,37,42,45]. The generic T cell response, or allergen-specific response when T cells recognize the allergen epitope, can instruct B cells towards IgE isotype switching in this compartment. In case of recognition by allergen-specific B cell receptors (membrane-bound immunoglobulin), these B cells can be selected to mature as IgE-producing plasma cells with cytokines inducing IgE isotype switching and co-stimulatory signals of neighboring allergen-specific Th cells and follicular Th cells. The T cell receptor (TCR) of these cells recognize the antigen that is presented via MHC-II by the B cells. Plasma cells will produce food-allergen-specific IgE [53].

Compartment 5: T and B cells homing towards tissue-specific ECM and epithelial barriers

T and B cells can home back to the lamina propria or dermis via the circulation (compartment 5) or affect distant sites (i.e., the gut or skin) [54]. Correct tissue homing to the gut requires α4β7 and CCR9 expression on effector T cells that develop in the MLNs while homing to the skin requires CLA and CCR4 or CCR10 expression in the SDLNs. This allows for migration towards CCL25 secreted by the epithelium in the gut or CCL17, CCL22, or CCL27 secreted by skin epidermal cells, respectively [55–58]. Subsequently, the Fc region of antigen-specific IgE binds the FcεRI receptor on the surface of mast cells and basophils that will be recruited to the lamina propria or dermis. In the design of an OoC model, use of protein or synthetic hydrogel migration channels would allow immune cells to migrate back to the epithelial tissues. More complex models could focus on the

incorporation of endothelial cells and flow in these channels, to mimic more closely the blood circulation.

Gut–immune–skin connection

To connect the two LN sites of food allergen sensitization, compartment 5 includes a migratory channel representing the vascularization towards either the lamina propria of the gut and/or the dermis of the skin. Connecting the two sites of allergen sensitization allows for uni- and bidirectional exchange of secreted cytokines, IgE, or translocating immune cells, mimicking the systemic circulation (Box 2 and Figure 3). In the scope of this review, further engineering details were left out, and can be found in recent comprehensive reviews [59,60].

Future patient-originated gut–immune–skin OoC models

Most OoC models make use of primary cells of healthy donors as well as immortalized cell lines. However, OoC models representing the gut–immune axis or skin–immune axis might partly under-represent specific genetic and/or phenotypic deviations in patient tissues such as for example, in allergies. To more closely mimic this, *in vitro* gut as well as skin models could be developed from patient-derived tissues. However, ethical issues together with the (timely and frequent) accessibility of patient gut and skin tissues and donor variation will be the largest limitation of such a model. In case of food allergy OoCs, allergen-specific immune responses could be generated using immune cells of allergic donor blood. With this, the patient's specific response to a certain allergen could be tested in a gut–immune–skin OoC model to validate the model or study immunomodulatory components. In addition to this, easier accessible primary or immortalized gut and skin cell lines could be used, while adding tissue related factors known to drive allergic sensitization. This combination of adaptations would contribute towards a more personalized OoC model.

Concluding remarks and future perspective

Despite the progress towards elucidating the mechanisms behind epicutaneous food allergen sensitization and development of food allergy unraveling the gut–immune–skin axis, there are still several knowledge gaps (see Outstanding questions). Advanced *in vitro* gut–immune–skin platforms may offer a contribution to our understanding and may help to predict the sensitizing allergenicity risk of for example novel food proteins.

Many of the immunocompetent gut and skin OoC models discussed already show promising results with respect to replicating their human counterparts. Future research improving advanced immunocompetent gut OoC, skin OoC, and gut–skin OoC models should focus on the incorporation of multiple immune cell types, specifically immune cell types such as LCs, DCs, T cells, B cells, and mast cells if it comes to studying allergic sensitization and the outcome of the effector response. To incorporate all cells, compartmentalization of the immune cascade, such as is present in the skin and gut with a separation between the inductive and effector side, could be the key to success. In addition, ECM characteristics (e.g., hydrogel composition, hydrogel stiffness, and immune-activating capacity) might support or hamper immune cell migration, the latter should be avoided. Furthermore, to be able to phenotype immune cells, they should easily be retrieved from the OoC devices as well as their secretagogues. This enables elucidating their functionality as characterized by the type of immune response in the device.

Including a tissue-specific LN compartment to facilitate crosstalk between migrating DCs or LCs and naïve adaptive immune cells, priming the development of effector cells that home via the blood stream to the tissue, would be a crucial next step. This would greatly expand the potential of gut–immune–skin OoC models towards analyzing intestinal as well as epicutaneous food

Outstanding questions

How can we mimic food allergen sensitization in *in vitro* models using (immortalized) cell lines while also taking care of personalized characteristics (e.g., atopic versus nonatopic individuals and adult versus infant cell phenotypes)?

Will we be able to recapitulate the whole immune cell cascade, starting from DC activation towards B cell priming and isotype switching to IgE, in one single *in vitro* OoC platform?

What is required for future *in vitro* gut–immune–skin axis OoC platforms to finally replace current food sensitization animal models?

To what extent is a complete immune system OoC needed to be able to connect the gut and skin?

If we will not be able to implement the whole food sensitization immune cell cascade in one OoC platform, which subsets of immune cells alone would then be sufficient to include in order to reliably predict food allergen sensitization?

To what extent are food matrix composition and processing of food allergens determinants of the outcome of the gut–immune–skin OoC results, to distinguish between allergenic versus nonallergenic food allergens (e.g., food heating, food protein glycation, food digestion, whole protein extracts, and single food allergens)?

allergen sensitization. Although challenging, some researchers have attempted to create a LN OoC [61,62] and bone marrow OoC [63]. Although not all biofabrication technologies are applicable yet, the fast-growing technology of the 3D bioprinting field will enhance the complexity needed in such a spleen- or LN-including gut–immune–skin OoC system as well as compartmentalization of the food allergen sensitization immune response.

Gut–immune–skin devices have the potential to be used in the future to gain a better understanding of the mechanisms involved in food sensitization, as well as in the prediction of sensitizing allergenicity of novel food proteins or to study therapeutic strategies. However, oversimplifying complex mechanisms in *in vitro* models could hamper physiologically relevant outcomes as many aspects of the intact organ and/or system are accounted for. As the technology field (e.g., bioprinting) is rapidly evolving, future models could expand their complexity, by, for example, integrating immune cell compartmentalization and different facets of the immune response as discussed in this review, but also to include variations in the microenvironment. This could involve adding different cell types that are present in the organ studied or vasculature, connecting different organs as well as to apply mechanical stresses, such as stretch and flow. However, mimicking the function of the organ should always be prioritized over the correct replication of the architecture of the organs studied. In short, the balance between the physiological complexity and experimental simplicity to reproduce meaningful data in a cost-effective and physiological realistic manner should be found.

Author contributions

R.J., J.W.M.d.K., and B.H. wrote the first draft of the manuscript. S.B-N, L.E.M.W., and R.M. reviewed/edited the manuscript. All authors read and contributed to the final version of the manuscript.

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Declaration of interests

J.G. is head of the Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science at Utrecht University and partly employed by Danone Nutricia Research B.V. The remaining authors declare no competing interests.

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