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Review

Translational Research Using Human Organoids: Integrating Multi-Organ Communication and Gene Regulatory Networks

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Abstract: Human organoids provide increasingly sophisticated models of tissue-specific physiology and disease, but their use in studies of endocrine communication and gene regulation remains limited. Here, we examine how organoid-based systems may be developed to study inter-organ endocrine networks, using the vitamin D–FGF23–PTH axis as a representative example. This axis spans the intestine, liver, kidney, bone and parathyroid gland, and integrates hormonal feedback with transcriptional control of defined target genes. We distinguish self-organizing organoids, organoid-on-a-chip platforms and multi-organ microphysiological systems, as these classes differ substantially in their capacity to support endocrine exchange between tissues. We then discuss the physiological functions such a model would need to reproduce, including feedback regulation of PTH, CYP27B1, CYP24A1 and FGF23. Although several tissue modules and coupled culture systems have been reported, important gaps remain in the maturation, functional competence and quantitative integration of kidney, bone and parathyroid compartments. We further consider how single-cell multi-omics and perturbation-based approaches can connect endocrine signals with cell-type-specific regulatory mechanisms. In this context, we distinguish descriptive co-expression patterns from transcription-factor regulons and experimentally validated regulatory interactions. We outline an evidentiary framework that links ligand–receptor signaling to transcription-factor activity, cis-regulatory elements, target-gene regulation and functional perturbation. Finally, we propose practical criteria for evaluating inter-organ gene regulatory network platforms, including tissue-module competence, directional coupling, feedback-loop closure, physiological concentration ranges, regulatory resolution and reproducibility. These considerations define the experimental advances needed before multi-organ organoid systems can yield mechanistic insight into endocrine gene regulation and its perturbation in disease.

Keywords: human organoids; organoid-on-a-chip; multi-organ microphysiological systems; gene regulatory networks; single-cell multi-omics; personalized medicine

1. Human Organoids as Advanced *In Vitro* Models for Biomedical Research

Human organoids are three-dimensional (3D) multicellular structures derived from human embryonic stem (ES) cells, human induced pluripotent stem cells (hiPSCs), or tissue-specific adult stem cells. These “mini-organs”

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recapitulate key aspects of native tissue architecture and function, providing a platform for studying human development, disease mechanisms, and drug responses [1,2].

The generation of organoids generally follows a stepwise process. First, pluripotent or tissue-specific stem cells are prepared, often derived from patient samples such as blood, skin, or surgically resected tissues. Second, directed differentiation is achieved by exposing cells to defined combinations of growth factors and signaling molecules, guiding them toward specific organ lineages. Third, cells are cultured within a 3D extracellular matrix (ECM) scaffold, such as Matrigel, which supports spatial organization and cell–cell interactions [3]. Under these conditions, cells undergo self-organization to form complex tissue-like structures. Finally, long-term culture with periodic medium replacement allows for maturation over weeks to months, resulting in physiologically relevant tissue models. Compared with conventional two-dimensional (2D) cultures, organoids more closely mimic in vivo cellular behavior and tissue organization [1,4]. Consequently, they have emerged as established tools for drug discovery, toxicity testing, and disease modeling.

1.1. Scope and Contribution of This Review

Recent reviews have examined the engineering of multi-organ chip platforms, the use of single-cell multi-omics for gene regulatory network inference, and functional genomic approaches in organoid systems. However, these areas are often considered separately. As a result, endocrine signals measured in coupled cultures and regulatory relationships inferred from single-cell datasets are not always linked through a clearly defined experimental framework.

This review brings these perspectives together [4–6]. We first distinguish organoids, organoid-on-a-chip platforms and multi-organ microphysiological systems (MPS), as these models differ in their capacity to reproduce communication between tissues (Section 1.2). We then use the vitamin D–FGF23–PTH axis to illustrate the physiological requirements of an in vitro endocrine model, including the feedback interactions that connect the intestine, liver, kidney, bone and parathyroid gland (Sections 2 and 2.1–2.4).

Finally, we discuss how inter-organ signaling can be related to cell-type-specific gene regulation. We outline a stepwise framework linking an endocrine ligand to receptor activation, transcriptional regulation and candidate cis-regulatory elements, and we discuss experimental criteria for evaluating these links (Sections 3.1 and 5). Throughout, we consider both the opportunities provided by current organoid-based systems and the limitations that need to be addressed before they can support mechanistic studies of endocrine gene regulation.

1.2. Model Classes and Nomenclature

The terms organoid, organoid-on-a-chip, and multi-organ microphysiological system (MPS) are sometimes used interchangeably, although they represent different experimental settings. This distinction is particularly relevant for studies of endocrine communication.

An organoid is a three-dimensional cellular structure that develops through self-organization of stem-cell-derived or tissue-resident progenitor populations within an appropriate extracellular matrix environment [1,4]. Depending on the tissue of origin and culture conditions, organoids can reproduce selected aspects of tissue architecture, differentiation, and function. However, conventional organoid cultures generally lack controlled fluid flow, defined tissue-to-tissue interfaces, and a shared circulation through which factors released by one tissue can influence another.

An organoid-on-a-chip places an organoid or organoid-derived tissue in a microfluidic device [7,8]. Perfusion, mechanical stimulation, and defined gradients can improve control of the culture environment and, in some cases, tissue maturation. However, a device containing only one tissue type remains a single-organ model.

A multi-organ MPS connects two or more tissue compartments through a shared or linked medium [8–10]. This arrangement can support exchange of secreted factors and metabolites between compartments, allowing directional tissue-to-tissue responses to be examined. Its usefulness depends on the maturity of each module, appropriate compartment scaling, compatible culture conditions, and sufficiently sensitive readouts.

Table 1 compares the three classes against the requirements of the network considered here.

Table 1. Capabilities of the three model classes against the requirements of a multi-organ endocrine axis.

Requirement	Organoid	Organoid-on-a-Chip	Multi-Organ MPS
Self-organized 3D tissue architecture	Yes	Yes	Yes, per compartment
Controlled perfusion and flow	No	Yes	Yes
Two or more distinct organ identities	No	No	Yes

Table 1. *Cont.*

Requirement	Organoid	Organoid-on-a-Chip	Multi-Organ MPS
Shared circulating medium permitting endocrine transfer	No	No	Yes
Compartment volume and cell-number scaling	Not applicable	Limited	Required
Can demonstrate inter-organ endocrine signaling	No	No	Yes
Supports cell-type-resolved gene regulatory network (GRN) inference	Yes	Yes	Yes, but cells per compartment limit power
Relative throughput	High	Moderate	Low
Principal limitation for the network in Section 2	No directional transfer between organs	Single tissue only	Scaling, medium compromise, assay sensitivity

Cell source should also be stated clearly. hPSCs include human embryonic stem cells and hiPSCs. Adult stem cell-derived organoids arise from tissue-resident progenitors, whereas patient-derived organoids retain features of the source tissue, including disease-associated somatic alterations. Patient-specific hiPSC-derived organoids retain the donor's inherited genome but do not necessarily preserve the somatic or epigenetic state of the original tissue. Engineered tumor-microenvironment models additionally include selected stromal, vascular, immune, or extracellular-matrix components.

For vitamin D metabolites we use vitamin D_3 (cholecalciferol) for the parent compound, $25(OH)D_3$ for 25-hydroxyvitamin D_3, and $1,25(OH)_2D_3$ for 1α,25-dihydroxyvitamin D_3, the hormonally active metabolite, throughout the text and both figures.

2. Reconstructing Inter-Organ Communication via Organoid-Based Microphysiological Systems

To illustrate the potential of human organoids in future research, we highlight the regulation of bone metabolism by vitamin D. vitamin D increases bone mass by maintaining a positive calcium balance through the promotion of intestinal calcium absorption and renal calcium reabsorption [11]. However, bone formation requires not only calcium but also phosphate; thus, this process must be integrated with FGF23-mediated phosphate regulation. Simultaneously, parathyroid hormone (PTH) acts directly on bone tissue to modulate both bone and calcium metabolism. vitamin D is thought to regulate bone metabolism through the synergistic action of these three factors.

The molecular basis of this network was significantly elucidated through the development of vitamin D Receptor (VDR) knockout models and the molecular cloning of the 25-hydroxyvitamin D_3 1α-hydroxylase gene [12,13]. These landmark studies established that while vitamin D signaling is central to mineral homeostasis, its interplay with FGF23 involves both VDR-dependent and independent pathways [14]. Furthermore, the circulating levels of these hormones are governed by mutual feedback loops that depend on the body's calcium status. Under calcium deficiency, vitamin D mobilizes calcium from bone tissue to normalize blood levels. While the biosynthesis of active vitamin D occurs in the renal proximal tubules, FGF23 is produced by osteocytes and PTH by the parathyroid glands. This vitamin D–FGF23–PTH network, driven by transcriptional feedback loops, constitutes a fundamental principle of endocrine regulation across multiple organs. Although these interactions are conceptually understood (Figure 1), detailed analysis of signal transduction between specific organs and the responses of diverse intra-organ cell types has remained challenging.

Flux arrows (blue block arrows) give approximate daily calcium movements in a healthy adult in zero calcium balance at a dietary intake of approximately 1000 mg/day: intestinal absorption ≈ 350 mg/day, digestive secretion ≈ 150 mg/day, fecal loss ≈ 800 mg/day, glomerular filtration ≈ 10,000 mg/day, tubular reabsorption ≈ 98% of the filtered load (≈9800 mg/day), urinary excretion ≈ 200 mg/day, and bone formation and bone resorption ≈ 500 mg/day each. The extracellular fluid (ECF) calcium pool is ≈ 900 mg, of which the serum pool is approximately one fifth. These are reference values under the stated conditions and vary with intake, age, vitamin D status and renal function; fractional intestinal absorption alone ranges from 20% to 60% [11]. The values on the block arrows are for calcium; the corresponding phosphate fluxes are given in Section 2.2.

Black arrows denote enzymatic conversion, comprising hepatic 25-hydroxylation by CYP2R1 and renal 1α-hydroxylation by CYP27B1. Colored arrows denote transport of a hormone between organs and are labeled with the species transferred: PTH (orange), FGF23 (green), and $1,25(OH)_2D_3$ (purple). These arrows indicate the route taken by each hormone; the direction of its action on each target organ is given in Table 2. FGF23 is shown arising from the osteocyte rather than from osteoblasts or osteoclasts. The gray dashed arrow denotes the calcium-sensing input from the ECF pool to the parathyroid gland. A key to all four arrow types is printed within the figure.

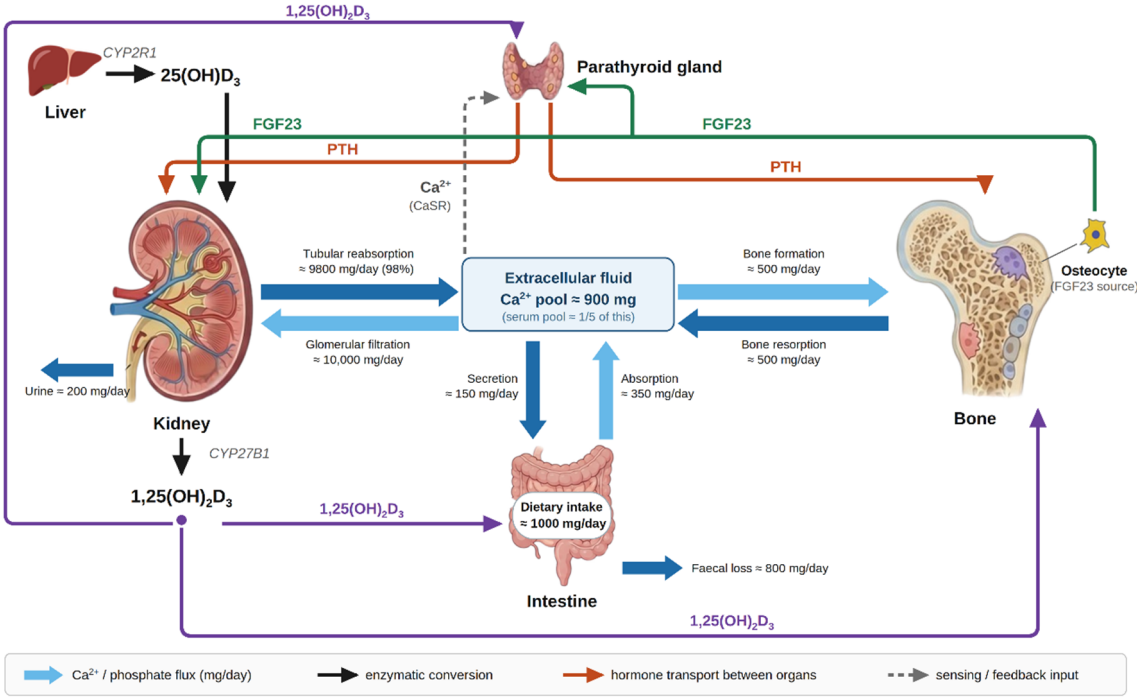

Figure 1. The vitamin D–FGF23–PTH network and calcium–phosphate fluxes in the healthy adult.

Abbreviations: CaSR, calcium-sensing receptor; CYP2R1, vitamin D 25-hydroxylase; CYP24A1, 25-hydroxyvitamin D 24-hydroxylase; CYP27B1, 25-hydroxyvitamin D_3 1α-hydroxylase; ECF, extracellular fluid; FGF23, fibroblast growth factor 23; FGFR1, fibroblast growth factor receptor 1; P_i, inorganic phosphate; PTH, parathyroid hormone; S100G, calbindin-D9k; SLC34A1/A3, NaPi-IIa/NaPi-IIc; SLC34A2, NaPi-IIb; TNFSF11, RANKL; TRPV5/TRPV6, transient receptor potential vanilloid 5/6; VDR, vitamin D receptor; 25(OH)D_3, 25-hydroxyvitamin D_3; 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3.

2.1. The Feedback Loops That Close the Circuit

An in vitro model that reproduced only the forward arms of this network would represent a signaling cascade rather than a regulated circuit. The interactions that close the circuit are therefore summarized below and are shown in Figure 1.

- Extracellular Ca^{2+} acts on the parathyroid calcium-sensing receptor to suppress PTH secretion, the fastest arm of the system [11].
- PTH induces renal CYP27B1, increasing 1,25$(OH)_2D_3$ production; the suppression of PTH by 1,25$(OH)_2D_3$ described below closes this loop [11].
- 1,25$(OH)_2D_3$ suppresses *PTH* gene transcription in the parathyroid. We have shown that liganded VDR represses the human *PTH* promoter through E-box-type elements, which provides a defined cis-regulatory endpoint for this loop [15].
- 1,25$(OH)_2D_3$ suppresses renal CYP27B1 and induces CYP24A1, limiting its own production and accelerating its catabolism [16].
- FGF23, acting on the kidney through FGF receptors that require α-Klotho as an obligate co-receptor, suppresses CYP27B1, induces CYP24A1 and reduces phosphate reabsorption; some of these actions are VDR-independent [14,17].
- FGF23 acts directly on the parathyroid to suppress PTH [18], while PTH in turn increases osteocytic *FGF23* expression, forming a bone–parathyroid feedback loop [19].
- 1,25$(OH)_2D_3$ and phosphate loading both increase *FGF23* expression [17].

Most of these interactions end in the transcriptional regulation of a defined gene, including PTH, CYP27B1, CYP24A1, FGF23, TRPV5, TRPV6, and SLC34A1/SLC34A3, and this property allows the endocrine description of the network to be related to its regulatory description, an approach developed further in Section 3.1. Two of them, though, are not transcriptional. Extracellular Ca^{2+} acting through CaSR suppresses the release of preformed PTH within minutes, and the fall in PTH mRNA that follows owes more to reduced transcript stability than to reduced transcription, so the measurement that matters is secreted PTH rather than PTH mRNA [11]. The acute

phosphaturic effect of FGF23 is likewise post-translational: FGFR1/α-Klotho signaling in the proximal tubule drives internalization and lysosomal degradation of apical NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3), and the fall in transcription follows more slowly [14,17]. Neither can be followed by measuring transcripts: the first calls for secreted hormone, the second for apical transporter abundance or transepithelial flux. The regulated targets of each hormone–organ pair, and whether these are transcriptional or transport targets, are summarized in Table 2.

Table 2. Transcriptional and transport targets of each hormone in each organ of the vitamin D–FGF23–PTH network.

Target Organ (Cell Type)	PTH	$1,25(OH)_2D_3$	FGF23	Ca^{2+}/Phosphate
Kidney (proximal/distal tubule)	↑ CYP27B1; ↑ TRPV5; ↓ SLC34A1/A3	↓ CYP27B1; ↑ CYP24A1; ↑ TRPV5	↓ CYP27B1; ↑ CYP24A1; ↓ SLC34A1/A3	—
Bone (osteocyte/osteoblast)	↑ TNFSF11 (RANKL); ↑ FGF23	↑ TNFSF11 (RANKL); ↑ FGF23	—	↑ FGF23 (phosphate load)
Parathyroid (chief cell)	—	↓ PTH transcription (VDR, E-box)	↓ PTH (FGFR1/α-Klotho)	↓ PTH secretion (CaSR)
Intestine (enterocyte)	—	↑ TRPV6; ↑ S100G; ↑ SLC34A2	—	—

↑ denotes induction or activation; ↓ denotes repression or inhibition; an em dash indicates no established direct action.

2.2. Calcium and Phosphate Fluxes

Figure 1 also places these interactions in the context of daily mineral fluxes. In a healthy adult in zero calcium balance, dietary calcium intake is approximately 1000 mg/day, of which roughly 350 mg/day is absorbed across the intestine; digestive secretion returns approximately 150 mg/day to the lumen, giving net absorption of approximately 200 mg/day and fecal loss of approximately 800 mg/day. The extracellular fluid (ECF) calcium pool is approximately 900 mg, of which the serum pool is roughly one fifth. Approximately 10,000 mg/day of calcium is filtered at the glomerulus and 98% of this is reabsorbed along the nephron, giving urinary excretion of approximately 200 mg/day, which balances net intestinal absorption. Bone formation and resorption each move approximately 500 mg/day and, in the steady state, cancel [11,20]. These are reference values under the stated conditions and vary with intake, age, vitamin D status and renal function; fractional intestinal absorption alone ranges from 20% to 60% [20]. Figure 1 states the units, the assumed intake and the source for every value shown.

Phosphate moves on a different scale and through different parts of the nephron. Intake is approximately 1000–1500 mg/day and about 65% of it is absorbed, mostly in the duodenum and jejunum; absorption is largely paracellular and follows the luminal concentration, with a smaller active component carried by NaPi-IIb (SLC34A2) and induced by $1,25(OH)_2D_3$. Net absorption of approximately 700–900 mg/day is matched by urinary excretion of a similar amount; the rest of the ingested load appears in the feces. Approximately 7000 mg/day is filtered, 80–90% of it is reabsorbed, and 60–70% of the filtered load is recovered in the proximal tubule through the apical cotransporters NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3). Bone accretion and resorption exchange on the order of 200–300 mg/day each. Roughly 85% of body phosphorus is held in bone mineral, so the extracellular pool is small by comparison with the skeletal reservoir it draws on [11].

The two minerals therefore ask different things of a renal compartment. Calcium is reabsorbed mainly in the distal nephron through TRPV5, and PTH and $1,25(OH)_2D_3$ increase it; phosphate is reabsorbed in the proximal tubule through NaPi-IIa and NaPi-IIc, and both PTH and FGF23 decrease it. A compartment reproducing distal calcium handling alone would say nothing about the phosphaturic arm, so a proximal-tubule-like phenotype is needed for the phosphate readouts as well as for CYP27B1-dependent 1α-hydroxylation. The fluxes drawn in Figure 1 are the calcium ones.

The application of human organoids now enables the replication of inter-organ signaling and the exchange of nutrients and metabolites—processes that are difficult to observe directly in the intact organism. This organoid-on-a-chip approach, and its multi-organ extension defined in Section 1.2, combines organoid technology with bioreactor systems and sophisticated co-culture methods (Figure 2), allowing researchers to recreate elements of the complex systemic environment of the human body [7]. Among these systems, kidney organoids present unique challenges due to the structural complexity of the nephron. Current protocols involve differentiating hiPSCs into intermediate mesoderm, followed by patterning into nephron-forming cells and collecting duct progenitors [21].

While 3D aggregation promotes the formation of glomeruli and renal tubules, achieving full structural and functional maturation remains a critical frontier in the field [22].

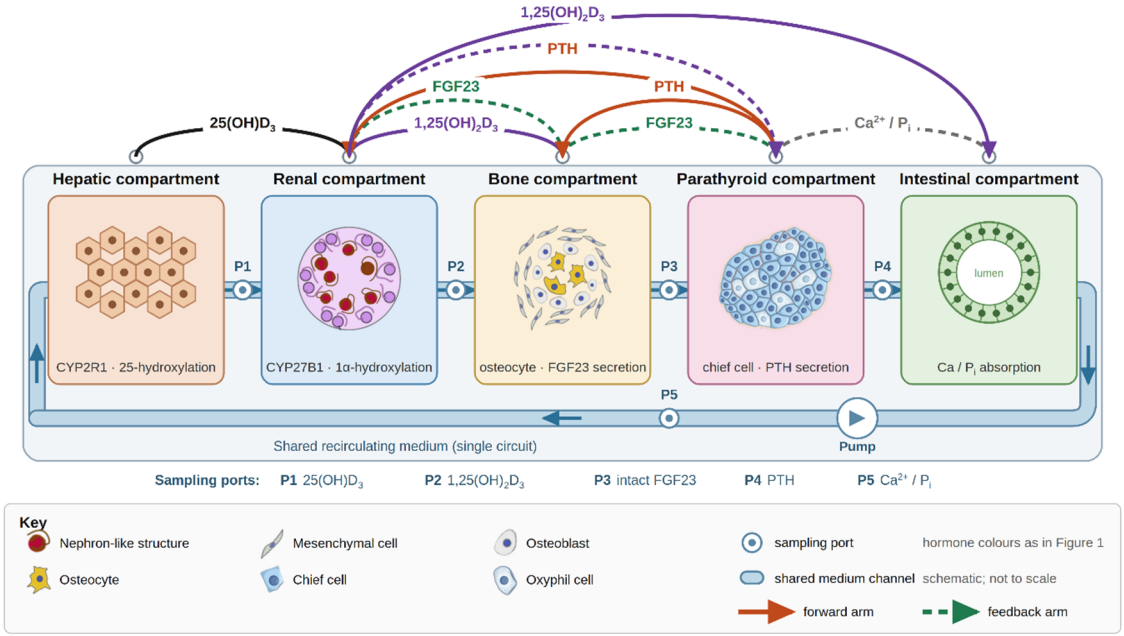

Figure 2. Proposed five-compartment microphysiological system for reconstructing the vitamin D–FGF23–PTH axis. This is a conceptual design. No device of this configuration has been reported, and the figure sets out requirements rather than describing an existing system.

Upper register, signaling topology. Nodes are aligned with the compartments beneath them. Solid arcs represent forward arms and dashed arcs represent feedback arms; each is labeled with the species transferred and colored as in Figure 1. The circuit is closed only when the four feedback arms are present, since a device reproducing the forward arms alone would represent a cascade rather than a regulated circuit (Section 2.1).

Lower register, device. Five tissue compartments are perfused in series by a single shared recirculating medium (blue channels, one pump): hepatic (CYP2R1-dependent 25-hydroxylation), renal (proximal-tubule-like cells, the intended site of PTH-inducible, CYP27B1-dependent 1α-hydroxylation), bone (osteocyte-containing, for FGF23 secretion), parathyroid (chief cells, for PTH secretion), and intestinal (calcium and phosphate absorption). Compartment volumes and cell numbers are set by allometric scaling (Section 4). Sampling ports P1–P5 indicate where medium is withdrawn, for $25(OH)D_3$, $1,25(OH)_2D_3$, intact FGF23, PTH, and calcium and phosphate, respectively. Cell types are identified in the key.

Absorptive surfaces. Each compartment is drawn as a single block, and the shared medium stands for the circulating, basolateral side alone; no luminal channel is shown. Absorption is nevertheless vectorial, and measuring it requires an epithelium whose apical and basolateral faces can be reached separately. The intestinal compartment is therefore taken to be a two-chamber culture (an epithelium on a porous membrane, or an apical-out organoid), with the apical surface facing a small luminal reservoir that carries the calcium and phosphate load and the basolateral surface facing the shared medium; absorption is then the transepithelial flux between the two, sampled at P5. The lumen drawn inside the intestinal organoid marks epithelial polarity and is not itself accessible for loading or sampling. The renal compartment has the same requirement, with the luminal side corresponding to the filtrate rather than to the circulation. In a single-chamber arrangement only the net change in medium calcium and phosphate can be measured, and apical uptake cannot be separated from basolateral exit.

Limitations of the design. Whether current human kidney organoids support PTH-inducible CYP27B1 activity and secrete measurable $1,25(OH)_2D_3$ remains unresolved (Section 2.4), and this is shown as a requirement of the renal compartment rather than as an established property. Where a hepatic compartment is omitted, $25(OH)D_3$ must be supplied exogenously (Section 2.4). Channel geometry, compartment shape, and device dimensions are illustrative and are not drawn to scale.

Abbreviations as in Figure 1; MPS, microphysiological system.

2.3. Current Multi-Organoid and Multi-Organ Chip Platforms

Several coupled multi-tissue systems have now been reported, and these provide a practical basis for assessing what such platforms can currently achieve. Human pancreatic islets and liver spheroids have been functionally coupled on a single chip, reproducing insulin-dependent glucose handling and providing an ex vivo model of type 2 diabetes [23]. A liver–islet multi-organoid system derived from hiPSCs has since reproduced comparable behavior in normal and diabetic states [24]. In a three-tissue liver–heart–lung platform, drug and metabolite effects were transferred between compartments, and cardiotoxicity was shown to arise from a hepatic metabolite rather than from the parent compound [25]. More recently, a multi-organ chip connected matured heart, liver, bone, and skin niches through a vascular flow compartment and maintained tissue-specific identity for four weeks [26]. The design considerations for such platforms, and the integration of organoids with microfluidic devices, have been reviewed elsewhere [8,10].

These studies indicate that a coupled vitamin D–FGF23–PTH platform is technically feasible, and they also indicate the remaining difficulties. None of the systems described above closes a hormonal feedback loop of the kind outlined in Section 2.1, and the bone compartment in ref [26] was not characterized for FGF23 output. The configuration shown in Figure 2 is therefore presented as a proposed design rather than as an existing device.

2.4. Representative Methods for Kidney, Bone, Parathyroid, Intestine and Liver Organoids

Kidney. In addition to the protocols noted above [21,22], perfusion in a millifluidic device has been shown to improve vascularization and maturation [27]. Two limitations are relevant here. Single-cell analyses indicate that kidney organoids correspond transcriptionally to first- and second-trimester fetal kidney rather than to adult kidney, and that approximately 10–20% of cells in several protocols represent off-target neuronal or muscle-like populations [28,29]. In addition, we are not aware of a published demonstration that a human kidney organoid expresses CYP27B1 in a PTH-inducible manner and secretes measurable $1,25(OH)_2D_3$. Until such data become available, the renal compartment is best regarded as an intended rather than a demonstrated site of active vitamin D synthesis, and Figure 2 is annotated accordingly.

Bone. Progress in this module has been more limited. Recent work has produced hiPSC-derived bone-marrow organoids containing stromal, vascular, and hematopoietic components [30], and a consensus effort has begun to standardize terminology, construction methods, and quality criteria for bone organoids [31]. A human osteocyte compartment that is embedded in a mineralized matrix, mechanically loaded, and competent to secrete FGF23 in response to PTH, $1,25(OH)_2D_3$, or phosphate is not yet routinely available. Since FGF23 is produced by osteocytes, this compartment is likely to determine the overall performance of the system, and osteocytes are therefore labeled explicitly in Figure 2.

Parathyroid. Two approaches have been described. Patient-derived organoids obtained from resected parathyroid adenoma retain PTH secretion and calcium responsiveness, and have been applied to tracer and drug screening [32]. hiPSC-derived parathyroid organoids have been reported to resemble parathyroid morphology and to secrete PTH [9], and construction methods have been compared systematically [33]. A recent transcriptomic analysis of hiPSC-derived parathyroid lineage cells, however, found that maturation was arrested at a parathyroid–thymic primordium stage without reaching adult chief-cell identity [34]. These observations are compatible, since hormone secretion is a less stringent criterion than adult transcriptional identity, but together they define the current limits of the model.

Intestine and liver. Both compartments are required for the circuit to close, and both were absent from our earlier schematic representation. The intestinal compartment contributes the calcium and phosphate absorption arm together with its VDR-dependent transporters, and intestinal organoids are among the better characterized systems currently available, including in chemically defined matrices [3]. The hepatic compartment contributes CYP2R1-dependent 25-hydroxylation. Where this compartment is omitted, $25(OH)D_3$ must be supplied exogenously, which is an acceptable simplification provided that it is stated explicitly.

3. Identification of Gene Regulatory Networks Underlying Diseases

The integration of single-cell and spatial multi-omics technologies represents a second direction. Endocrine organoids exhibit considerable cellular heterogeneity, reflecting the diversity of cell types within native tissues. Single-cell approaches enable the identification of cell-type-specific transcriptional programs and regulatory elements, while spatial transcriptomics provides insights into the spatial organization of signaling interactions [5]. These technologies are particularly valuable for studying endocrine cell differentiation, lineage trajectories, and niche-specific gene regulation, and community efforts to establish shared organoid reference atlases provide one approach to improving comparability between laboratories [35].

Another major frontier lies in the application of CRISPR–Cas genome editing to interrogate gene regulatory mechanisms. Organoids derived from hPSCs or patient-specific hiPSCs provide a suitable platform for functional genomics. Targeted perturbation of transcription factors, enhancers, and signaling pathways enables the causal investigation of gene regulatory circuits [36–39]. For instance, CRISPR-mediated disruption of lineage-specific transcription factors has provided mechanistic insight into lineage specification. This has been shown most clearly in human brain organoids, in which multi-omic profiling identified candidate transcription factor–element–gene relationships and subsequent CRISPR perturbation confirmed the predicted changes in cell fate [36]. Combining CRISPR perturbations with single-cell transcriptomics and chromatin accessibility profiling (e.g., scRNA-seq and scATAC-seq) allows for high-resolution mapping of regulatory networks at the cellular level. At genome scale, Perturb-seq has been used to resolve regulatory relationships across thousands of genes [37], and pooled screens in human intestinal and colon organoids have identified drivers of TGF-β resistance and tumor suppressor genes, respectively [38,39].

3.1. From an Inter-Organ Signal to a Causal Gene Regulatory Network

The approaches described above provide the necessary measurements. Using them to explain how an endocrine signal reaches a particular gene is a further step, and one that depends on what kind of evidence is being offered.

Co-expression is the weakest form. Genes whose expression covaries across cells or conditions may or may not be under the control of the same factor, and in organoid datasets the covariance is often driven by differences in cell-type composition rather than by regulation within a cell type. A transcription-factor regulon is a stronger statement, since it assigns genes to a factor on the basis of motif enrichment in accessible chromatin together with expression correlation, as implemented in SCENIC+ for paired single-cell transcriptome and accessibility data [40]. It nevertheless remains an inference. Motifs are shared within transcription-factor families, so the responsible member is frequently unresolved, and accessibility does not establish occupancy. What matters for mechanism is whether perturbing the regulator, or the element itself, produces the predicted change in the target gene in the relevant cell type. In silico perturbation methods such as CellOracle are useful for deciding which links are worth testing in this way [41], but the test still has to be carried out.

Set out in full, the path from an inter-organ signal to a validated regulatory event runs from the circulating factor and its receptor, through the signaling output and the transcription factor it engages, to the cis-regulatory element that factor occupies, the gene that element controls, and finally to a perturbation that tests the link. Written in this form, the sequence appears more orderly than it is in practice, and it is worth being explicit about where the difficulty actually lies.

The first two elements are usually the best established. For FGF23 acting on the kidney the receptor complex is FGF23 together with FGFR1 and α-Klotho [17], which has the practical consequence that α-Klotho expression in the receiving compartment must be confirmed before any response is interpreted, since a compartment lacking α-Klotho will not respond at all. For PTH acting on the proximal tubule the route through parathyroid hormone 1 receptor (PTH1R) and cAMP/PKA is similarly well characterized, and for $1,25(OH)_2D_3$ the ligand-activated VDR–RXR heterodimer acts on chromatin directly, so no intermediate needs to be identified.

The account becomes weaker where the transcription factor is connected to a specific regulatory element. In most published work this connection is inferred from the presence of a motif, or transferred from data obtained in non-human cell lines, rather than measured in the system under study. Linking that element to the gene it controls is a related problem, and in practice the two are often not separable: an element identified only by its proximity to a promoter carries little information about which gene it regulates. Chromatin contact data and expression responses are required for both.

Perturbation is the step that converts the account from an inference into a claim. It is also the step most often omitted, since deleting or silencing an individual element in a differentiated three-dimensional culture is considerably more demanding than profiling one.

Within the present network the PTH locus is the most tractable starting point, because receptor, element, and target gene are all specified in advance: we have previously mapped VDR-responsive E-box elements in the human PTH promoter [15]. CYP27B1 is more demanding but more informative, since PTH on the one hand and $1,25(OH)_2D_3$ and FGF23 on the other drive it in opposite directions, and a method that cannot recover that opposition is unlikely to be reliable elsewhere. FGF23 in osteocytes provides a third case, responding to PTH [19], $1,25(OH)_2D_3$, and phosphate, although it is the least accessible experimentally for the reasons given in Section 2.4.

3.2. Current Limitations and Challenges

Several constraints apply when organoids are used to identify gene regulatory networks.

Sparsity and dropout in single-cell data reduce the power to detect regulatory relationships involving transcription factors expressed at low levels, a group that includes many of the factors of interest here. Motif-based assignment is often unable to distinguish between paralogous family members. Benchmarking of inference methods also remains incomplete, since reported performance varies with the dataset, the definition of ground truth, and the evaluation metric applied; an inferred network is therefore best regarded as a hypothesis conditioned on the method used. Cell-type composition differs between organoid batches, and because most inference methods aggregate across cells, such differences can generate apparently distinct networks from biologically comparable material. Metabolic and endoplasmic reticulum stress can also distort cell identity in organoids and confound subtype assignment [42]; this was described in cortical organoids, although the underlying mechanism is unlikely to be organ-specific. Finally, pooled perturbation screens require a sufficient number of cells per perturbation to achieve adequate statistical power, which is difficult to obtain for a rare cell type such as the osteocyte within a small multi-organ compartment.

4. Clinical and Therapeutic Applications of Human Organoids

Patient-derived organoids (PDOs) are valuable tools for modeling disease-specific gene regulation and advancing personalized medicine [43]. hiPSC-derived organoids retain the donor's genetic background, allowing researchers to investigate how genetic variation influences endocrine signaling and transcriptional responses; as noted in Section 1.2, these organoids differ from patient-derived organoids, which retain the somatic genome of the source tissue. Organoids derived from patients with metabolic disorders or endocrine tumors can reveal disease-specific alterations in gene expression. These models are also well suited to high-throughput drug screening and to evaluating therapeutic interventions in a patient-specific context. Integrating organoid models with clinical data and machine learning is being explored as a route to identifying predictive biomarkers and optimizing treatment strategies [44,45].

In oncology, the 3D architecture and the tumor microenvironment (TME) are central. Organoid systems allow for the incorporation of ECM components [3], and, in engineered tumor-microenvironment models, of mechanical cues and stromal interactions, all of which influence signaling pathways and transcriptional regulation. Compared to 2D cultures, 3D systems better reproduce the signaling molecule gradients and physical constraints that shape cellular behavior. Despite these advances, challenges remain: current systems often lack full vascularization, immune components, and long-term maturation [27]. Ongoing efforts to incorporate endothelial cells, immune populations, and perfusable vasculature are expected to enhance the physiological relevance of these models.

The preceding paragraph refers to three limitations. Several others are relevant to the claims that can reasonably be made from these systems.

Organoids of most lineages remain transcriptionally closer to fetal than to adult tissue [28]. Undefined extracellular matrix preparations introduce lot-to-lot variability, which chemically defined matrices are intended to reduce [3]. Establishment success rates and batch-to-batch variability are reported inconsistently, and cross-site reproducibility has been assessed formally for only a small number of organoid types [5,6]. Clinical evaluation of organoid-guided drug response has so far been carried out in defined settings and cohorts [43,44], and these results do not yet support routine use for treatment prediction; a recent appraisal of organoid readiness for drug discovery reaches a comparable conclusion [45].

Three further constraints apply specifically to coupled multi-organ systems. The first concerns scaling. Compartment volumes and cell numbers must be chosen so that a hormone secreted by a small compartment reaches a physiologically meaningful concentration in the shared medium; without explicit allometric scaling, the concentration experienced by a receiving compartment may differ from the human range by several orders of magnitude. The second concerns medium compatibility. Each organ module has its own optimal culture medium, and a common medium represents a compromise that may itself alter transcriptional state. The third concerns materials and readouts. Polydimethylsiloxane absorbs small hydrophobic molecules, including steroid-derived ligands such as $1,25(OH)_2D_3$, and the sample volumes available are often below the sensitivity of standard immunoassays for PTH and intact FGF23. These are tractable engineering problems, but they determine whether an observed coupling reflects physiology or the properties of the device.

5. Outlook: What Would Need to Be Demonstrated

What would need to be shown before a coupled system of this kind could be used to draw conclusions about endocrine gene regulation? Table 3 lists six requirements together with our reading of how far current systems meet them. Two of the six carry most of the weight.

The first is that each compartment actually performs the regulated function of the organ it represents. For the renal compartment this means PTH-inducible *CYP27B1* and measurable $1,25(OH)_2D_3$ in the medium; for the bone compartment, osteocytic *FGF23* that responds to PTH and to phosphate; for the parathyroid compartment, PTH secretion that is suppressed by calcium. None of these has been demonstrated in the configuration proposed here, and until they are, coupling the compartments together is unlikely to produce interpretable data.

The second is loop closure. A system in which a step change in medium calcium or phosphate moves PTH, FGF23, and $1,25(OH)_2D_3$ in the directions set out in Section 2.1, rather than moving one of them, is doing something that separate cultures cannot do. This is the point at which the added complexity of the platform becomes worthwhile.

Table 3. Requirements for an inter-organ gene regulatory network platform reconstructing the vitamin D–FGF23–PTH network, with our assessment of the current position.

	Requirement	What Would Need to Be Shown	Current Position
1	Module competence	Renal: PTH-inducible *CYP27B1* with measurable $1,25(OH)_2D_3$ output. Bone: osteocytic *FGF23* responsive to PTH and phosphate. Parathyroid: calcium-suppressible PTH secretion.	Not met for the renal or bone compartment; partly met for the parathyroid compartment [9,32,34]
2	Directional coupling	Perturbation of one compartment changes a named transcript in another; the change is abolished by receptor blockade or knockout in the receiving compartment.	Not yet demonstrated for this network
3	Loop closure	A step change in medium Ca^{2+} or phosphate changes PTH, FGF23, and $1,25(OH)_2D_3$ in the directions predicted in Section 2.1.	Not met
4	Quantitative range	Hormone concentrations in the medium fall within reported human physiological ranges, and the allometric scaling calculation is stated.	Not reported
5	Regulatory resolution	Paired single-cell transcriptome and accessibility data per module; TF–element–gene links nominated computationally, then perturbed.	Achieved in single-organ systems [36]; not yet in coupled systems
6	Reproducibility	Reproduced across at least three independent donors or cell lines and three independent experiments, with analysis code and primary data deposited.	Not established

The remaining requirements are less discriminating but still necessary. Directional coupling has to be shown to depend on the receptor, most simply by blocking or deleting it in the receiving compartment. Hormone concentrations in the shared medium should be reported against human reference ranges with the scaling assumption stated; this is routinely omitted, and without it a measured response cannot be placed on a physiological scale. Regulatory resolution, in the sense of Section 3.1, has so far been achieved in single-organ systems [36] but not in coupled ones. Reproducibility across donors and independent experiments is a general requirement of the field rather than a feature of this network, but it is worth restating here, since the batch variability discussed in Section 3.2 bears directly on network inference.

6. Conclusions

Human organoids have made it possible to monitor organ functions and inter-organ communication “live” within a defined human cellular environment. This capability offers a new route to the study of gene regulation and signaling. By enabling organ-level analysis of genetic diseases caused by genomic mutations and epigenetic alterations in chronic conditions, human organoids provide a framework for elucidating disease progression and providing new strategic directions for therapy and drug discovery. Applying this approach to a specific endocrine network will require more than the coupling of organoids. It will also require compartments that demonstrably perform the regulated function of the corresponding organ, a closed rather than a linear circuit, hormone concentrations within the physiological range, and regulatory links that are tested by perturbation rather than inferred alone. Current models meet some of these requirements but not others, and the preceding sections indicate where the principal gaps remain.

Author Contributions

Each author is expected to have made substantial contributions to the conception or design of the work; or the D.M.L.: conceptualization, investigation, visualization, writing—original draft preparation, writing—reviewing and

editing; Y.K.: investigation; T.K.: investigation; S.K.: conceptualization, supervision, project administration, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

Given the role as Editor-in-Chief of *Gene Regulation and Signaling*, Shigeaki Kato had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

AI-assisted tools were used for language editing; the authors reviewed and take full responsibility for the content.

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