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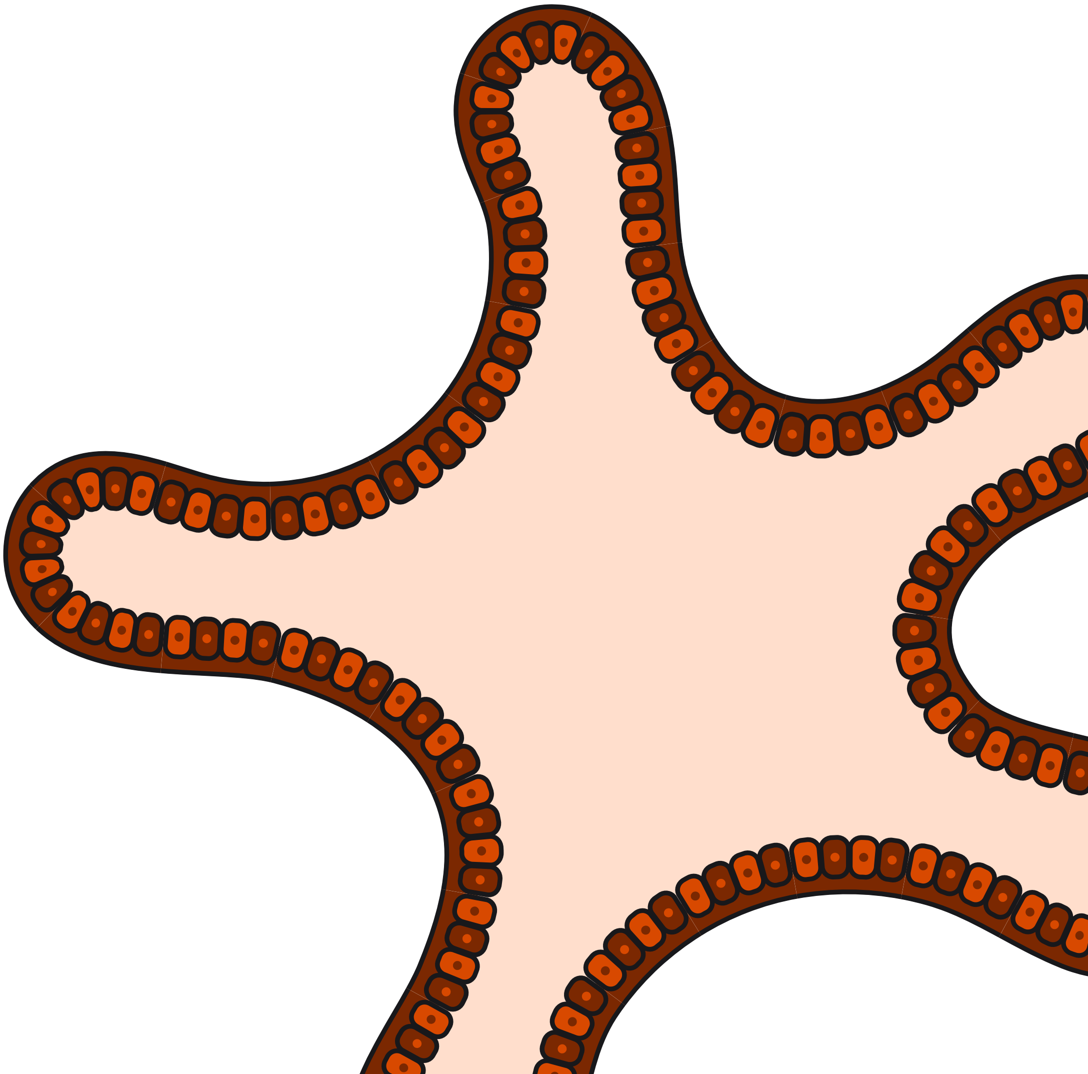
Ready to demystify organoids?

Discover the top five applications for organoids.

Plus, we cover a typical workflow, five common challenges, and dissociation methods.



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Onward research areas utilizing organoid technology

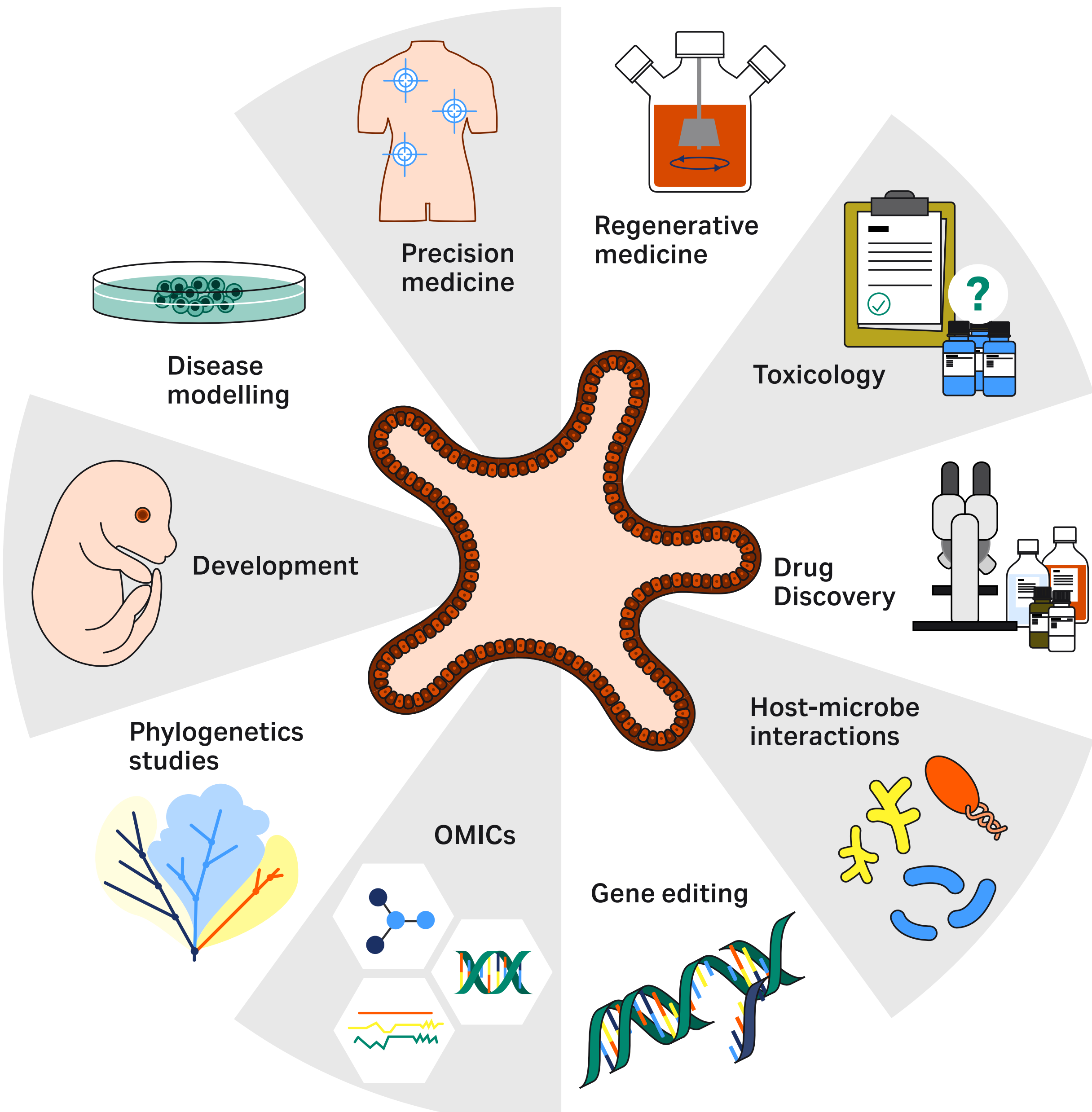

Fig 1. Illustration of the onward uses of organoids.

Patient-derived organoids and their uses

Patient-derived organoids have several important uses in biomedical research and personalized medicine. Here are a few key applications:

1. **Disease modelling:** Organoids derived from patient tissues can provide a more accurate representation of specific disease conditions compared to traditional cell cultures or animal models. Researchers use these organoids to study various diseases such as cancer, neurodegenerative disorders, and gastrointestinal conditions. By replicating the characteristics and behavior of the patient's organs (Figure 2), scientists gain insights into disease progression, test potential treatments, and identify personalized therapies. Recent advancements in research technology have led to the development of "fashionable models" including organoids and organ-on-chip (OoC) models. These provide reliable safety and efficacy assessments for therapeutics without animal testing. Click here to learn more about fashionable models in drug discovery

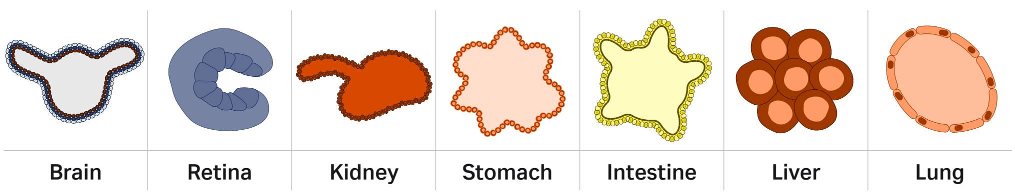

Fig 2. Illustration (only) of organs represented by organoids.

2. **Drug discovery and development:** Organoids are valuable tools for drug screening and testing. Pharmaceutical companies use organoids to assess efficacy and toxicity of potential drugs before moving to clinical trials. By testing drugs on patient-derived organoids, researchers can better predict how an individual might respond to a specific drug, enabling more personalized treatment strategies.

3. **Precision medicine:** Patient-derived organoids can be used to develop personalized treatment plans. By analyzing the genetic and molecular characteristics of an individual's organoids, doctors can identify specific therapies that are most likely to be effective for the specific patient. Organoid-based precision medicine has the potential to improve patient outcomes, minimize adverse effects, and reduce treatment costs.

4. **Biomarker discovery:** Organoids derived from patient samples can help identify specific biomarkers associated with disease progression or treatment response. By analyzing the gene expression, protein profiles, and drug sensitivity of organoids, researchers can discover new biomarkers that may aid in early diagnosis, prognosis, and monitoring of diseases.

5. **Regenerative medicine:** Organoids can be used to develop and test regenerative therapies. By culturing and manipulating patient-derived stem cells within organoids, researchers can study tissue development, regeneration, and potential transplantation strategies. Organoids help advance the field of regenerative medicine by providing a platform to test safety and effectiveness of tissue-engineered constructs.

While patient-derived organoids have shown great promise, further research is still needed to fully understand their potential and address any limitations.

Typical workflow

The workflow for generating patient-derived organoids generally involves several key steps. Here is a typical workflow:

1. **Patient sample acquisition:** The process begins with obtaining a tissue sample from the patient. This can be done through a biopsy, surgery, or other methods depending on the organ of interest. The sample is collected and transported to the laboratory under appropriate conditions to maintain its viability.

2. **Tissue processing:** Once the sample arrives at the laboratory, it's processed to isolate the relevant cells. The tissue is typically washed, minced, and enzymatically digested to break down the extracellular matrix and release individual cells or organoid-forming units.

3. **Organoid formation:** The isolated cells are then embedded in a three-dimensional matrix, such as Matrigel or a synthetic hydrogel, that provides structural support. The cells are cultured in specialized media containing growth factors and nutrients that promote organoid formation and maintenance. The cells self-organize and differentiate to form organoid structures that closely resemble the tissue of origin.

4. **Organoid growth and maintenance:** The organoids are cultured in a controlled environment, such as an incubator, to support growth and maturation. The culture media are regularly replenished with fresh nutrients and growth factors to sustain organoid development. The culture conditions, such as temperature, humidity, and oxygen levels, are optimized to mimic the physiological environment of the organ.

5. **Characterization and analysis:** The generated organoids are then characterized using various techniques to confirm their identity, structure, and functionality. This can involve histological staining, genetic analysis, immunofluorescence, or functional assays.

Specific details of the workflow may vary depending on the organ of interest (Figure 3 describes customer organoid workflow) and the research objectives. Additionally, advancements in organoid culture techniques and automation are continually streamlining workflows for generating patient-derived organoids.

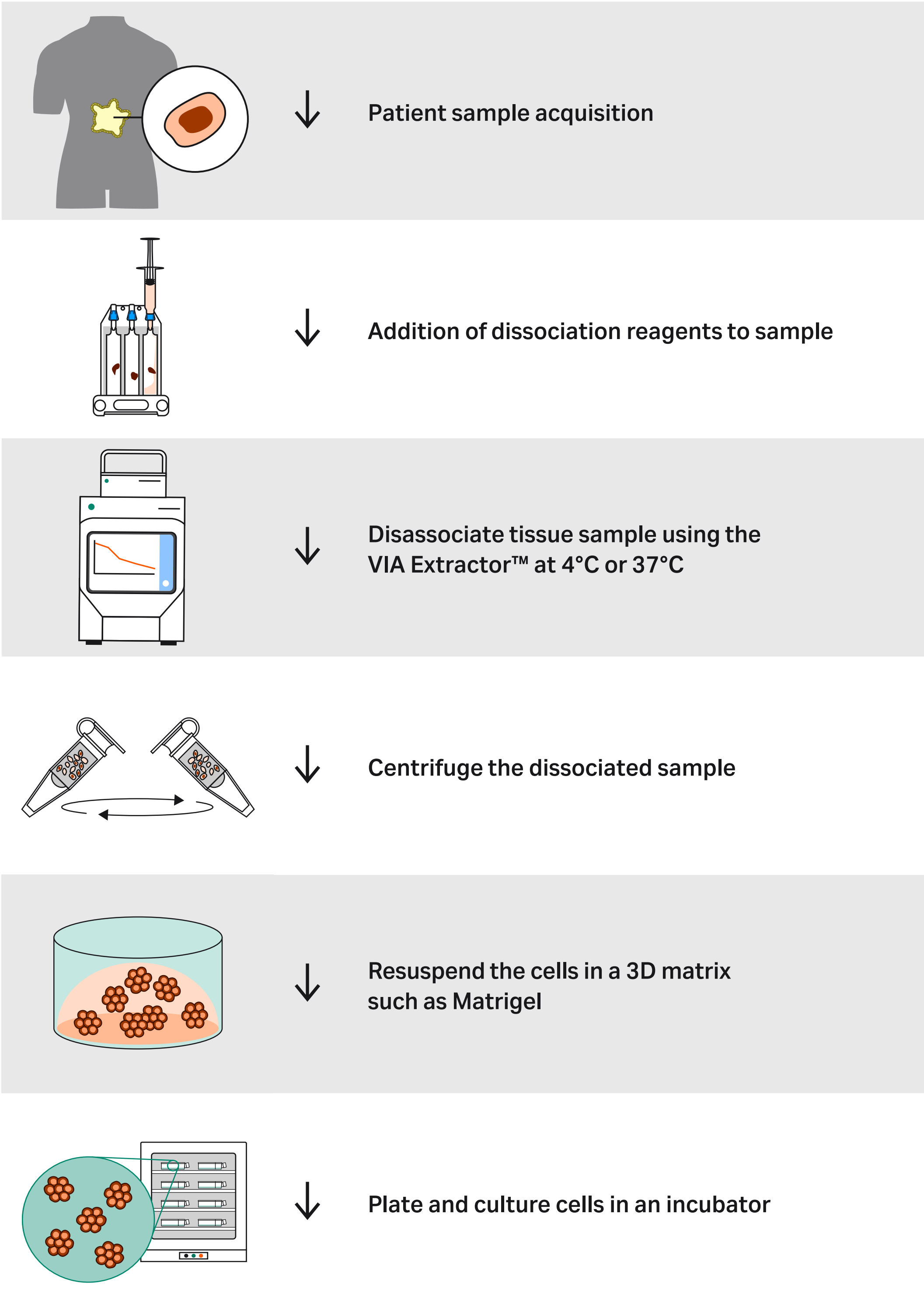

Fig 3. Customer dissociation workflow for the development of intestine organoids.

What are the challenges in current dissociation methods?

Tissue dissociation, the process of breaking down tissue into individual cells or organoid-forming units, can pose several challenges when generating patient-derived organoids. Here are some common challenges associated with tissue dissociation:

1. **Cell viability:** The dissociation process can be stressful to cells, potentially affecting their viability and functionality. Factors such as enzymatic digestion time, temperature, and mechanical forces used during dissociation can impact cell viability. Maintaining high cell viability is crucial to ensure the successful formation and growth of organoids.

2. **Tissue heterogeneity:** Patient tissues can be highly heterogeneous, containing various cell types with different properties and behaviors. Achieving a uniform dissociation of the tissue into single cells or organoid-forming units can be challenging. Some cell types dissociate more readily than others. This leads to variations in the cell composition and quality of the resulting organoids.

3. **Loss of cell specificity:** During tissue dissociation, cells can lose their original identity and functionality. The dissociation process disrupts the natural cell-cell and cell-matrix interactions, potentially altering gene expression patterns and cell behavior. It is important to optimize dissociation protocols to minimize the loss of cell specificity and preserve the characteristics of the original tissue.

4. **Contamination:** Tissue dissociation can introduce contamination from non-target cell types, such as immune cells or fibroblasts. These contaminating cells may interfere with the growth and function of the organoids or affect downstream analyses. Proper purification techniques, such as cell sorting or selective culture conditions, may be necessary to minimize contamination and maintain the purity of the desired cell population.

5. **Reproducibility:** Achieving consistent and reproducible tissue dissociation can be challenging, particularly when working with different patient samples or tissue types. Variations in tissue characteristics, such as tissue density, extracellular matrix composition, and cell-cell adhesion properties, can affect the efficiency of tissue dissociation. Optimization and standardization of dissociation protocols are necessary to ensure reproducibility across experiments.

Researchers are continually working to address these challenges by developing improved dissociation techniques, optimizing protocols, and exploring alternative strategies to enhance the generation of patient-derived organoids. These efforts aim to improve the reliability and fidelity of organoid models for biomedical research and clinical applications.

How do you dissociate organoids?

Dissociating organoids typically involves breaking down the three-dimensional structure of the organoids into individual cells or smaller organoid fragments. The specific methods employed for dissociation can vary depending on the organoid type and the research objectives. Here are a few common approaches used for organoid dissociation:

1. **Chemical dissociation:** Involves the use of chemical agents to disrupt cell-cell adhesions and release the cells from organoids. Chemical dissociation is often used in combination with enzymatic or manual methods to enhance dissociation efficiency. One example is a chelating agent like ethylenediaminetetraacetic acid (EDTA) which is used to weaken calcium-dependent cell-cell adhesions.

2. **Enzymatic dissociation:** Involves treating the organoids with enzymes that break down the extracellular matrix and disrupt cell-cell adhesions. Commonly used enzymes include collagenase, trypsin, dispase, or accutase. The organoids are incubated with the enzyme solution for a specific duration, usually at an optimized temperature, to allow the enzymes to break down the matrix and release the cells. Commonly used with automation such as the VIA Extractor tissue disaggregator for controlled speed of motion, temperature and duration to achieve optimal results. Figure 4 describes common procedure using the VIA Extractor tissue disaggregator for organoid dissociation. Figure 5 shows VIA Extractor tissue disaggregator in use the in the laboratory.

Fig 4. Examples of VIA Extractor tissue disaggregator in use in the laboratory.

The best choice of dissociation method depends on factors such as the organoid type, tissue characteristics, desired cell viability, and downstream applications. Optimization and careful validation of dissociation protocols is crucial to minimize cell stress, maintain cell viability, and preserve the functional properties of the dissociated cells for subsequent experiments.

Genomics specialists at Cytiva help customers create an optimal protocol for their sample, including organoids, to ensure your precious samples maintain optimal cell viability.

Find out more about the VIA Extractor tissue disaggregator

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