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Sensing technologies and experimental platforms for the characterization of advanced oral drug delivery systems

Peter Ouma Okeyo [a,*], Sriram Thoppe Rajendran [a,b], Kinga Zór [a,b], Anja Boisen [a,b,*]

[a] Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics, Department of Health Technology, Technical University of Denmark, 2800 Kongens Lyngby, Denmark
[b] BioInnovation Institute Foundation, 2200 Copenhagen, Denmark

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ABSTRACT

Complex and miniaturized oral drug delivery systems are being developed rapidly for targeted, controlled drug release and improved bioavailability. Standard analytical techniques are widely used to characterize i) drug carrier and active pharmaceutical ingredients before loading into a delivery device (to ensure the solid form), and ii) the entire drug delivery system during the development process. However, in light of the complexity and the size of some of these systems, standard techniques as well as novel sensing technologies and experimental platforms need to be used in tandem. These technologies and platforms are discussed in this review, with a special focus on passive delivery systems in size range from a few 100 μm to a few mm. Challenges associated with characterizing these systems and evaluating their effect on oral drug delivery in the preclinical phase are also discussed.

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* Corresponding authors.

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1. Introduction

Drug discovery and development of oral dosage forms is a time consuming and complicated process. In the preclinical phase, [1-3] there is a preformulation step [4] in which the physical, chemical, and mechanical properties of the active pharmaceutical ingredient (API) are determined using a toolbox of analytical techniques [5]. With the results obtained in the preformulation stage, a solid form of a drug can be recommended. The physical, chemical, and mechanical properties of the API should be well understood to ensure the successful development of the formulation.[6-8] Conventional formulations are widely used for oral drug delivery (ODD), and this route of delivery is the most commonly used method of drug administration. This is because of the ease of administration and patient compliance [9-11]. However, there are certain limitations with conventional formulations, such as the need for repeated dosing frequency, and poor aqueous solubility, which results in low oral bioavailability. [12-14].

In the 1950s, drug delivery systems (DDSs) were introduced to improve the performance of many existing drug products (DPs) such as cyclosporin (Neoral®), ritonavir (Norvir®), and saquinavir (Fortovase®) with the use of lipid-based DDS (LBDDS). [12,15-17] This resulted in a flurry of new therapies [18], some of which employed miniaturized, μm and mm scale oral drug delivery systems (ODDSs) to enable a more targeted approach [19-25].

It is essential to define what constitutes an active ingredient versus a DP in the context of his review. The US Food & Drug Administration (FDA) defines the final DP as a dosage form. For example, tablet, capsule, or solution containing the API generally, but not necessarily in association with inactive ingredients [9-11]. In this review, a drug delivery system (DDS) is defined as a combination of a device and its loaded contents (i.e., API and excipients/polymers).

Different micro and nano fabrication techniques have facilitated the growth of miniaturized oral drug delivery devices [26], primarily because these techniques enable precise control in size and shape. With this level of control, it becomes possible to create customized drug delivery devices using various materials. This facilitates the development of these devices, including; diverse shapes, topologies, compartments (reservoirs), and sizes (nm to mm) with the aim to improve oral drug delivery. Considering the substantial variation in the designs (Fig. 1) microelectromechanical systems (MEMS) technologies have and continue to play an important role in the fabrication of these devices [27-29].

In general, DDSs are divided into two categories: passive and active. Passive DDSs release the API by simple diffusion over time from their reservoir. Reservoir-based DDSs may have a simple device structure and implementation [26]. On the other hand, active DDSs are considered more complex, in structure, with the ability to alter the device's passage and control the release of API [38-41].

Overall, there is extensive literature focusing on the fabrication of ODD microdevices [42-44], and there is an emerging trend in the development of mm-sized devices for oral drug delivery [24,45-47]. However, there are a limited amount of articles describing the characterization of oral drug delivery devices and the encapsulated/loaded API. In this review, challenges in the characterization of ODDSs, focusing on the loaded API, excipients, and the ODD are highlighted. Moreover, we discuss new techniques, sensors, and experimental platforms used to solve these challenges. This review is structured in the following way; **a)** standard characterization techniques in preclinical development, **b)** novel, purpose-developed assays, sensors, and bioanalytical techniques in preclinical development, **c)** and future perspectives. The passive devices that will be discussed will range from micro (few 100 μm) to mm-sized ODDSs. For a broader perspective, the reader is directed to reviews where liposomes, microparticles, and nanofibers have been extensively discussed by others [45 48]. Additionally, active ODDs such as micromotors have been published [49,50].

2. The development cycle of drug delivery systems

Fig. 2 shows examples of novel ODDSs and some of the most commonly used traditional and novel characterization methods used when investigating differently shaped and sized drug delivery devices. Here, the methods and techniques are briefly described in the context of the development cycle of ODDs. Sensing methods and techniques will be discussed in more detail in the following sections.

Fig. 2a depicts an example of a novel oral drug delivery device by Grayson et al. [51]. The authors demonstrated the application of a biodegradable poly (L-lactic acid) device that releases an API called dextran over several months. To achieve this, a complex design was used that consisted of multiple reservoirs. The additional complexity of this device is due to the multiple considerations during the design process, such as the thickness of the device, material, molecular mass of the loaded API (and its excipients), and the stability of the loaded formulation. As a quality control step, the authors, during the fabrication steps, frequently checked (quality control) the device to ensure they were defect-free, then loaded the API.

The characterization of the API may be performed before loading as a quality control step, as suggested in Fig. 2b. This can be normally performed using standard analytical techniques such as microscopy, X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) [60], thermogravimetric analysis (TGA), and spectroscopic methods [5]. More recently, MEMS technologies have been employed for characterizing pure (without impurities) [60] samples, where the sample is deposited on the surface (string resonators) or inside a resonator (membrane resonators). Studies have shown superior sensitivity of these resonators, with the advantage that as little as pico-gram amounts of material being used for analysis [61]. An added benefit of characterizing both small and large compounds (~50–1500 Dalton) is the ability to probe the molecular fingerprint of the API [62]. MEMS devices have also been used to characterize amorphous compounds and proteins [63]. Recently, it was demonstrated that these measurements could be performed by using drug particle (polycrystalline) resonators [52]. Once the API and/or polymers are well characterized, they can be loaded into a drug delivery device.

Different methods are used for loading [26] small and macromolecules into the delivery devices. Fig. 2c shows five examples, where the API or formulation has been loaded into the device using; inkjet printing [53], supercritical CO_2 impregnation [55], manual filling [64], powder embossing [65], as well as spin coating, and hot punching [56]. With each method, different considerations need to be taken to ensure that the device is sufficiently filled [66,67], with the desired solid form of the API. This is important

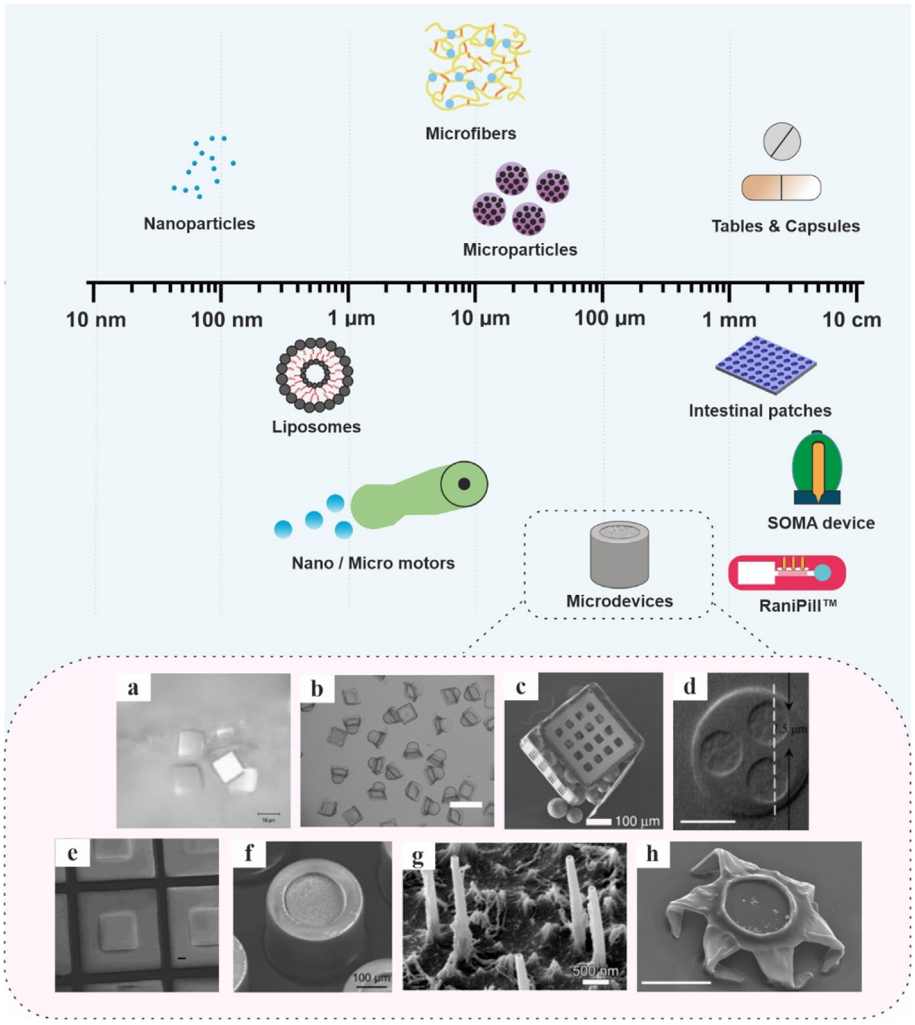

Fig. 1. The size scale of drug delivery systems. The inset shows oral drug delivery devices of different topography, shape, and size. **a)** 50 μm microdevice (scale bar = 50 μm) [30], **b)** 50 μm microcapsules (scale bar = 50 μm) [31], **c)** 500 μm self-loaded microcontainer with 150 μm glass beads (scale bar = 100 μm) [32], **d)** 200 μm circular device with three 60 μm circular reservoirs (scale bar = 100 μm) [33], **e)** 150 μm hydrogel-loaded microdevice (scale bar = 20 μm) [34], **f)** 270 μm microcontainer filled with an amorphous sodium salt of furosemide (scale bar = 100 μm) [35], **g)** 2.2 μm long nanostraws (scale bar = 500 nm) [36], **h)** 150 μm theragrippers in closed form with the ability to unfold with an overall size of 250 μm (scale bar = 100 μm) [37]. Figures a) – g) reprinted with permission from respective publishers and f) shared under a Creative Commons Attribution 4.0 International License.

because uncontrollable or undesired phase changes (process and/or solvent-induced transformations) can occur in the API depending on the loading method [68,69]. The use of XRD and thermal techniques would be helpful in not only performing a quality control step of the API before and after and develop experimental platforms that can monitor phase transitions occurring during loading.

Imaging is frequently used to visualize the contents of the DDSs, but this can be increasingly challenging for devices that are thick, opaque, and/or hold large reservoirs. For example, with confocal Raman microscopy, the imaging depth is normally limited to a few microns as seen in Fig. 2d. Here an example of 2D Raman imaging of a DuoCaplet device is shown [57]. Other techniques such as X-ray micro-computed tomography (μ-CT), can also be used to image and visualize the different layers of the formulation. The benefit here is that it is possible to use μ-CT imaging to see deep into materials. However, it does not give any structural information [70], which can be critical if phase transitions are already occurring in the drug. This can happen prior, during, and/or after the loading step. Other 3D imaging methods have been explored and will be discussed in a later section in this review.

Fig. 2e shows a planar microdevice that delivers the API, using nanostraw membranes [71]. The nanostraws are used to facilitate a tunable release of API and improve the device's bioadhesion properties. The benefit of this device is linked to the added nanoscale features, however, these features may be challenging to fabricate [71,72]. The authors used confocal fluorescence imaging and SEM to visualize the overall device structure, loaded API, and the integrity of the nanoscale features. It is apparent that these features are fragile, and mechanical tests would need to be performed. This would determine if these structures could retain their integrity in biological media and the needed force for sufficient binding onto the lining of the small intestine. Shear stress tests were performed using a flow cell with a solution of porcine mucin that passed through the flow cell [73]. It was determined that 76% of the drug delivery devices had a strong adhesion to the mucosa compared to a control.

There has been a notable increase in the development of novel *in vitro* characterization platforms. These platforms can be used to predict the *in vivo* response of the API. Fig. 2f shows a poly (lactic-co-glycolic acid) (PLGA) thin film (100 μm) device that was designed with varying ratios of lactic and glycolic acid to release resolving D1 (RvD1) to targeted tissues. [58] The goal of this ODDS was to deliver sustained (over 14 days) and unidirectional release of API in injured tissues. Lance et al [58]used a variety of standard

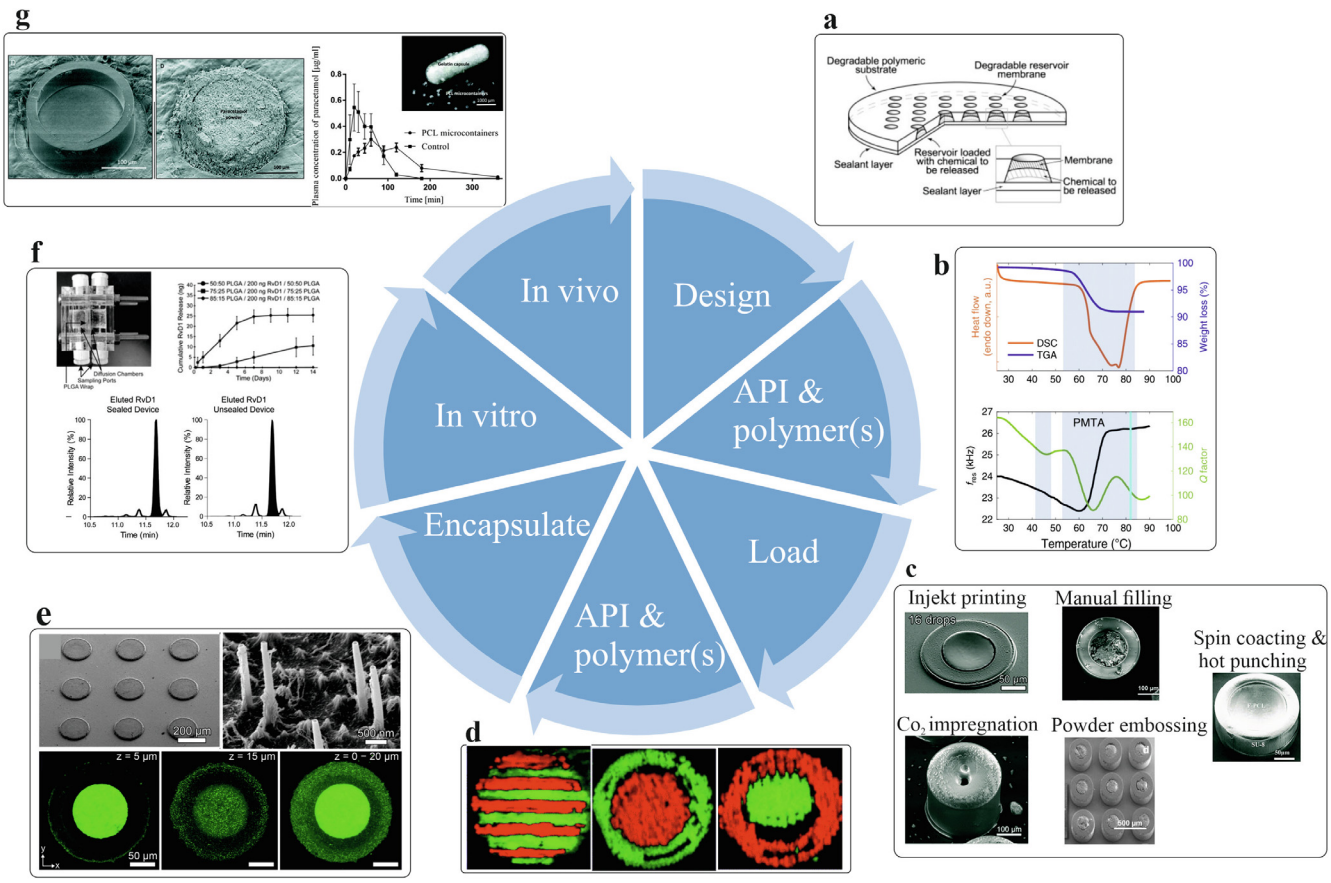

Fig. 2. Examples of oral drug delivery systems (ODDSs) and standard as well as novel methods and techniques used for the handling, characterization, and evaluation of these systems in the development process. a) design of a polymeric device with multiple compartments for release over long periods [51], **b)** comparison between differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and particle mechanical thermal analysis (PMTA) [52], **c)** different methods of loading API & polymers into the drug delivery devices, where inject printing was used to load 10 mg/mL of insulin (scale bar = 50 μm), CO_2 impregnation (200 bar 4 h) for filling ketoprofen (scale bar = 100 μm), manual filling of amorphous indomethacin in a 174 μm diameter microcontainer (scale bar = 100 μm), powder embossing of lipid based microparticles (scale bar = 500 μm), spin coating and hot punching a formulation of furosemide and poly- ε-caprolactone in a photoresist SU-8 and biopolymer poly-l-lactic-acid (PLLA) microdevice (scale bar = 50 μm) [53-56], **d)** 2D-Raman imaging of a multilayer DDS where the multilayer device contains 8.2% paracetamol-PVA with 9.5% caffeine-PVA, caffeine-PVA (core) where caffeine - green, paracetamol – red [57], **e)** DDD with nanostraw structures. Scanning electron microscopy (SEM) of drug delivery devices with nanostraw membranes and confocal fluorescence images (below) of the device structure showing drug reservoir, overlaying membrane and device structure respectively (devices were incubated at night over 4 °C in 10 mg/mL fluorescein isothiocyanate -bovine serum albumin (FITC-BSA) solution [36], **f)** Schematic of a multi compartment DDS showing the PLGA wrap, diffusion chambers and sampling ports, release results for 50:50 PLGA/200 ng RvD1/50:50 PLGA, 75:25 PLGA/200 ng RvD1/75:25 PLGA and 85:15 PLGA/200 ng RvD1/85:15 PLGA, and where 50:50 PLGA side shows faster release, LC-MS results of eluted sealed and unsealed device with RvD1 [58], **g)** single empty PCL microcontainer and loaded with paracetamol, image of a gelatin capsule with multiple PCL containers, comparison between the plasma concentration of control and PCL microcontainer with paracetamol over 300 min, mean ± SEM n = 7 with microcontainers and n = 8 with control [59]. Figures a), c) -g) reproduced with permission from respective publishers. Figure b) and a section of figure c) is shared under a Creative Commons Attribution 4.0 International License. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

analytical techniques to characterize the device, including elution studies with enzyme immunoassay kits, stability studies in various chemical solutions, liquid chromatography-mass spectrometry (LC-MS), to evaluate the structural integrity of RvD1 inside and once released in the target tissue. The use of orthogonal techniques in this study provided adequate information to be confident in the stability of the API and the DDS. Previous attempts to deliver RvD1 locally and over a long period have been met with challenges that are linked to cytotoxicity.[74-76]

Abid et al. [59] demonstrated the application of biodegradable microcontainers made of poly-ε-caprolactone (PCL) as shown in Fig. 2g. Here a μ-dissolution setup was used for *in vitro* characterization of drug release, as well as several orthogonal analytical techniques such as scanning electron microscopy (SEM), μ-CT, and high-performance liquid chromatography (HPLC) before *in vivo* studies in rats. The *in vivo* results suggested a higher bioavailability compared to conventional dosage forms. [77]

There have been examples of ODDS that have reached phase I of clinical trials, for example, RaniPill™, developed by RaniTHERAPEUTICS. [78] RaniPill™ is a capsule device with a microtablet. [79] Phase I trials have been performed to assess the safety, tolerability, and performance of RaniPill™ in 58 healthy volunteers using octreotide (100 μg). The results were published in 2020 and showed 70% oral bioavailability with no serious adverse events. [80] This device shows the potential for ODDS to progress from drug discovery to clinical development.

3. Techniques to evaluate the structural characteristics, topology, retention, and mechanical properties of the drug delivery devices

As mentioned in the introduction, traditional microfabrication methods are suitable for engineering drug delivery devices. [27,117] Microfabrication facilitates the development of specially designed and fabricated drug delivery devices that could be used to address typical challenges of oral drug delivery, such as safeguarding the API [64], facilitating mucoadhesion [36], enabling sustained [33] and/or unidirectional release [114] and improving

Table 1
Summary of the most relevant and currently used methods for characterization ODDSs in the previously mentioned size range. These will be discussed in more detail in the following sections.

Test parameter	Description	Techniques/methods	References
Morphology and topography	Used for observing: 1) Physical dimensions and geometric features immediately after fabrication, 2) Filling of drug/formulation into the reservoir, 3) Coatings/Encapsulation after drug filling 4) Emptied reservoir	Visual Optical microscopy Fluorescence microscopy Scanning electron microscopy (SEM) Optical profilometry Micro-computed tomography (μCT)	[30,31,34,46,53,59,81-84]
Particle size	Particle size and shape of solid particles	Dynamic light scattering (DLS) Polarized light microscopy (PLM) Laser diffractometry	[85-87]
Physical and chemical stability	Polymorphism and the investigation of phase transitions in API and the DDS	X-ray powder diffraction (XRPD) Hot stage microscopy (HSM) Differential scanning calorimetry (DSC) Thermogravimetric analysis (TGA) Dynamic vapor sorption (DVS)	[5,64,88-96]
Mucoadhesion	Crucial parameter for anchoring/tethering of devices in the GI tract	Tensile (detachment) method Flow-through method Rotating cylinder method	[97-103]
Mechanical characterization	For testing highly customized mechanical features of the device	Single device force measurement Finite element method (FEM)	[24,37,104]
Drug release and efficacy	Used for studying: Biocompatibility/Toxicity Release of drugs from devices Bioavailability of drugs released from the devices	*In Vitro* microDiss /UV absorption Caco-2 cell culture monolayer (static) / Transwell Caco-2 cell culture monolayer (flow) Microfluidics *Ex vivo* 1) Franz cells 2) Ussing chamber *In Vivo* 1) Animal models 2) *In situ* perfusion	[34,36,58,66,101,103,105-116]

bioavailability [35]. With increased complexity and variations in the size of these devices [24,25], characterization becomes crucial for developing suitable fabrication methods, for quality control during production, and, performance. [71] In Table 1, some key parameters and experimental methods frequently used for characterizing these drug delivery devices are presented.

3.1. Structural and topological characterization methods

SEM is the most commonly used characterization technique [31,101,118,119] immediately/or during fabrication, as a quality control step, to check the morphology of the fabricated devices (Fig. 3a1-a3). Additionally, atomic force microscopy (AFM) is also a powerful tool for studying the fabricated finite structures (Fig. 3b). [119] Particularly for morphology and topology characterization, in few cases, a simple bright-field microscope could also be employed (Fig. 3c) in combination with vertical scanning interferometry (Fig. 3d). The former provides the shape/size integrity and can estimate the dimensions of the drug delivery devices, while the latter can provide a surface profile in the μm range. [31]

Recently, micro-computed tomography (μ-CT) has also emerged as a valuable technique for the characterization of microdevices. For example, Mazzoni et al. employed the μ-CT to check the uniformity of polymeric coatings on the microdevices, which is otherwise not possible to characterize using SEM (Fig. 3e). [101]

3.2. Evaluation of the effect of topology on retention and mucoadhesion

The geometry of the delivery device can alter/prolong the resident time in specific regions in the gastrointestinal (GI) tract [102]. This could benefit the local drug absorption at the target sites. [120] In order to increase the net contact area with the intestinal lining, microfabricated patch systems have been proposed previously. [120,121] These patches are flat, thus reducing the area exposed to the flow in the intestinal lining, thereby further enhancing the resident time in the GI tract. [34] Extensive literature is available for characterizing intestinal patches' mucoadhesive and mechanical properties [122-125] and has been previously reviewed by Kirsch et al. [126]. Several other design features such as micro wells/reservoirs are also available with different geometries [72] that have the possibility of altering mucoadhesion through chemical and physical approaches.[34]

Previously, Tao et al. demonstrated the use of silane chemistry for surface modifying microdevices with tomato lectin for improved mucoadhesion. [119] This study used a monolayer of Caco2-cells as a model for the intestinal epithelium to evaluate mucoadhesion. While using a monolayer of Caco2-cells is highly standardized and widely accepted for drug transport studies, the cell monolayers are still far from the physiological conditions for reliable evaluation of mucoadhesion in the GI tract. Later, Lee and co-workers reported a dual-sided mucoadhesive and omniphobic device, with the aim to orient one side of the device towards the GI tract and the other towards the lumen [98]. They used Carbopol, a commercially available mucoadhesive polymer for the mucoadhesive side of the device. Carbopols mucoadhesive properties were evaluated with a flow retention system similar to the setup depicted in Fig. 4a. The experimental setup consists of porcine intestine tissues mounted over a slope, using a protocol and setup first reported by Rao and Buri in 1989. [127]

The precise structures on the surface of the microdevices lead to enhanced retention and even anchoring of the devices into the mucous layer in the GI tract. [128] For instance, Guan et al. employed a bilayer system of a poly(EGMA–co-EGDMA) and

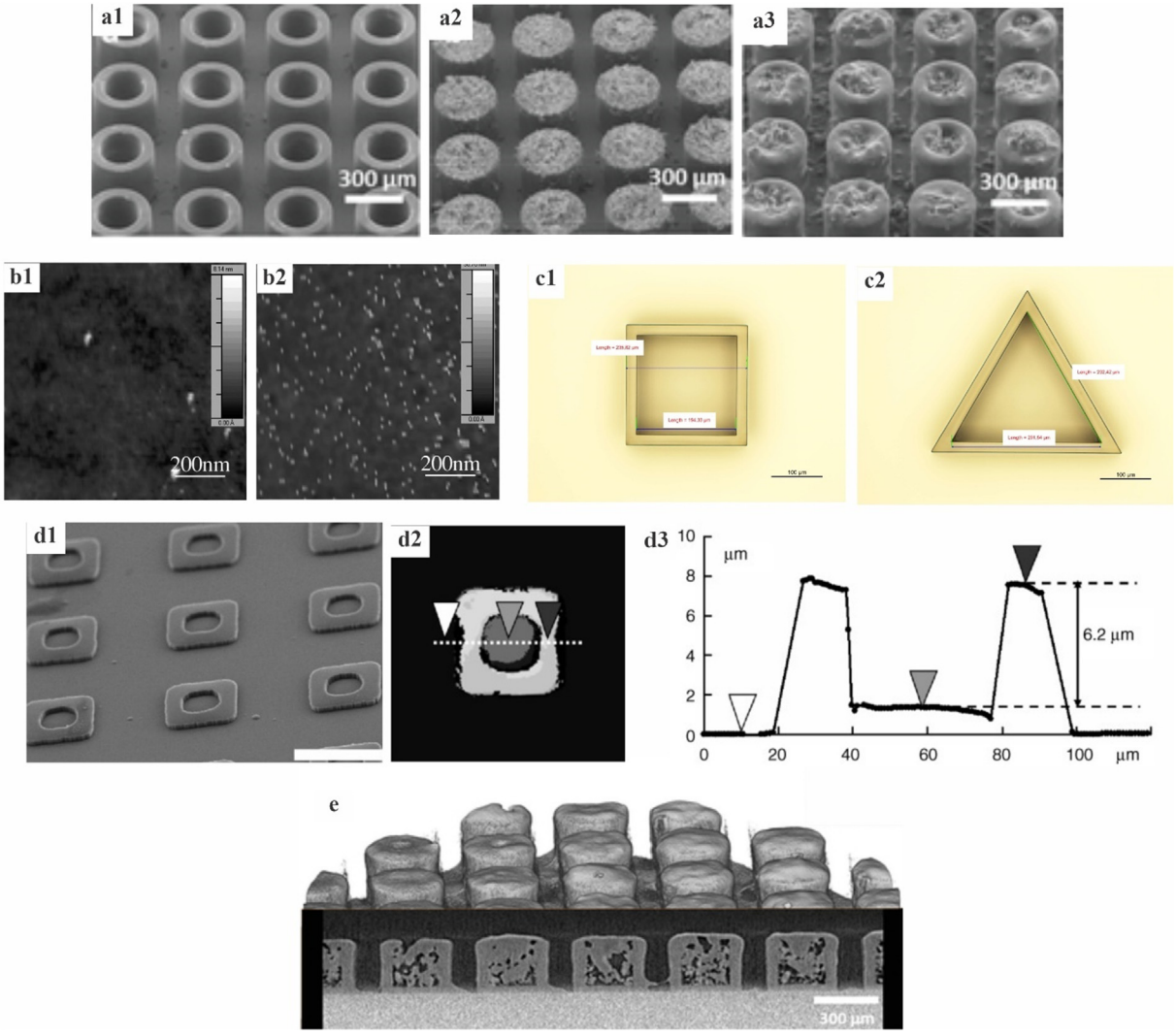

Fig. 3. Topography characterization of microdevices for oral drug delivery. SEM images of **a1)** empty microcontainers, **a2)** microcontainers filled with lysozyme and C10 (7:3 w/w), **a3)** filled containers encapsulated with PLGA [101], **b1)** AFM (1 µm × 1 µm) tapping mode images of, **b1)** unmodified and **b2)** modified PMMA [119], optical micrographs of microcontainers of different shapes: **c1)** square and **c2)** triangle [81], **d1)** SEM image of PVA microstructures, **d2)** and **d3)** cross-sectional profile of PVA microstructures [31],**e)** (µ-CT) of a cross section of an array of drug loaded microdevices with PLGA encapsulation on top of the reservoir [101]. All figures reproduced with permission from respective publishers.

cross-folding chitosan with self-folding arms intending to tether to the mucous layer physically. [31] They used a simple method for mucoadhesion studies by releasing the devices onto a wet porcine tissue placed over a glass slide. After rinsing, they counted the adhered devices on the tissue. However, they were not able to appropriately quantify the percentage of retention of these self-folding devices. In another study, Ghosh and co-workers proposed a bio-inspired therapeutic gripper (theragripper) using a novel design approach to enhance GI retention [37]. This design was inspired by a hookworm called *Ancylostoma duodenale,* which is known to reside in the GI tract of the human intestine for up to two years. Their device consisted of multiple claws with sharp microtips that enabled the devices to latch on the GI tract. [37] For successful adhesion, the force exerted by this device should be greater than the strong shear force generated due to GI peristalsis. Due to the small size of theragrippers, it was found challenging to experimentally measure the force exerted by the claws of the theragrippers. Using finite element method, they successfully estimated the force generated during claw folding and concluded that the claws could exert sufficient force to penetrate the mucosal barrier. To the best of our knowledge so far, such single device force characterization has only been performed for comparatively larger devices due to practical limitations in performing these experiments [37,104].

From the above examples, it is demonstrated that, unlike the topology characterization techniques, experimental techniques for the characterization of mucoadhesion are far from standardized. To reliably evaluate the mucoadhesion of ODDSs, suitable experimental platforms are required for faster iterative testing and re-design cycles. For this purpose, the use of *in vivo* studies is neither economically feasible nor ethically viable. Currently, the retention model by Rao and Buri [127] is widely used for the characterization of DDSs for *in-situ* bioadhesion (Fig. 4a). One of the major pitfalls of using such retention models is the lack of reproducibility of experimental conditions, mainly arising because of the custom-made experimental platform. Specifically, lack of control over humidity, temperature, simulated biological fluids, and flow rate negatively impacts the experiment's reproducibility. [97] More recently, Vaut and co-workers reported a fully replicable open-source experimental platform (Fig. 4b) for the characteriza-

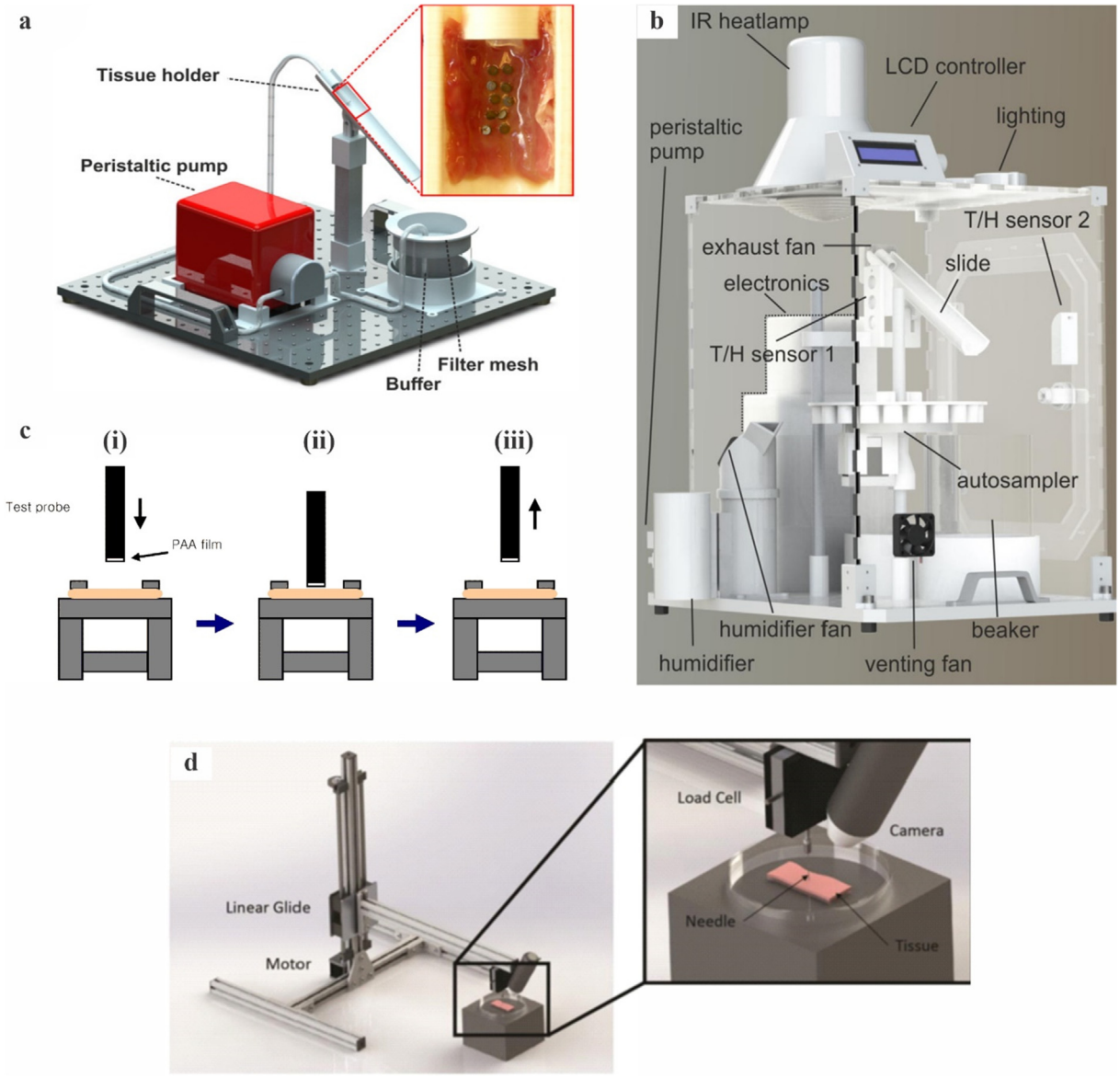

Fig. 4. Mucoadhesion charecterization of devices for oral drug delivery. a) Computer aided design of experimental setup for evaluating mucoadhesion using the flow retention setup first proposed by Rao and Buri [102,127], **b)** 3D rendering of a fully replicable automated retention measurement setup for characterization of bioadhesion [129], **c)** schematic representation of mucoadhesive test using texture analyzer using the tensile detachment method, **i)** probe with hydrated poly(acrylic acid) (PAA) film was moved downward, **ii)** dried PAA film was attached to porcine buccal mucosa, **iii)** the probe is withdrawn at specified rate [99], **d)** schematics of needle insertion mechanism. The device consists of a linear glide, stepper motor, 0.5 N or 10 N load cell and video camera. The inset shows the load cell attached to the device. All of the devices were controlled via a custom-made LabView setup. All figures reproduced with permission from respective publishers.

tion of bioadhesion with the aim of standardization. [129] The testing platform consists of temperature and a humidity-controlled chamber, a peristaltic pump, a tube-like tissue holder, and autosampler rotation for collecting the samples flown over the immobilized tissue. This setup is mainly fabricated using 3D printing, laser cutting, and using off-the-shelf electronic components. The simplicity of the setup enables other researchers to easily replicate the described system, thereby improving the reproducibility of experimental conditions.

To evaluate the strength of mucoadhesion, texture analyzers (or modified microforce balances) (Fig. 4c) have emerged as a standard tool for various DDSs [97,99]. Typically, the tested devices (singular or in an array) are fixed to a probe holder and brought in contact with the mucosa of excised porcine tissue for a defined period. Subsequently, the greatest force required to break the mucoadhesive bonding is determined. Based on the outcome, a force-distance plot is used for calculating the mucoadhesive force. The direction of separation of the device could further classify it as rupture, shear, and detachment force. [130,131].

Recently, an ingestible self-orienting millimeter-scale applicator (SOMA) for insulin delivery in the stomach was reported. The device contains insulin milliposts which orients towards the stomach wall and injects insulin via the milliposts. These milliposts have to be characterized for tuning the insertion force for the successful delivery of the payload. [24] Therefore, for this specific requirement, a custom-made setup was developed to measure the insertion force of insulin milliposts in the stomach lining of swine tissue, as shown in Fig. 4d. In yet another study, Prausnitz et al. had to characterize the force of microneedle-based patches. [25] In this case, the aim was to optimize the microneedle penetration just enough to penetrate the intestinal epithelium, thereby avoiding full-extent tissue penetration.

3.3. Characterization of biodegradable materials

With conventional formulations, there are various coating processes, for example, spray drying or film coating, that can be applied to tablets. Coatings can be applied for different reasons such as: to cover an unpleasant taste or odor, protection of the API, ensure the stability of the drug product, alter the API release pattern or protect the API from acidic conditions in the stomach. [132,133] The intended purpose, choice of coating, and characterization method are essential, especially if the coating is hygroscopic or temperature-sensitive. [134]

Numerous optical techniques are used to determine the surface properties of biodegradable coatings. Optical techniques such as dual polarization interferometry (DPI), brewer angle microscopy [135], surface plasmon resonance (SPR), and quartz crystal microbalance (QCM) [136], measure real-time changes occurring on the surface of DDS. However, the challenge of DPI and SPR techniques is their limited sensitivity, in particular, their limited ability to determine the structural integrity of the DDS once adsorbed on a surface, i.e., intestinal lining. This can be less of an issue with QCM.

On the other hand miniaturized blue-ray setup has also been repurposed to perform micromechanical characterization of PLGA in combination with a disposable microfluidic chip to better understand biopolymer degradation mechanisms and significantly speed up the experimental time from 6 weeks to 8 h. [137] This is done by tracking the resonance frequency and quality factor of PLGA coated micro resonators in varying enzyme conditions. Such a technique may be useful in evaluating the choice of polymers for drug delivery devices as well as gaining a more fundamental understanding of mechanical changes in the polymer.

4. Analytical techniques and sensing technologies used to investigate APIs

During the compilation of this review, it was apparent that there are limited published studies that perform/document a comprehensive characterization of the formulation prior to loading or when the API is in the delivery device.

The authors of the review propose that the properties of API and polymers, which can be crucial in identifying, quantifying intermediates, degradation products, impurities, and polymorphic forms should be characterized already before loading (in device). [7 88] Therefore, this section will discuss the various analytical techniques used to characterize the API and polymers at several stages: prior to loading, when the API is in the delivery device during storage, and after release.

4.1. Physical characterization of API before loading in the drug delivery device

There are generally three different levels of characterization, molecular (crystal structure), particulate (ensemble of particles), and bulk (averaged response of measured particles). [5] When performing solid-state characterization of the API in drug discovery, more specifically preformulation [4], determining the most stable solid form of an API is an essential step [90,138]. This is because most APIs can form polymorphs [139] that may affect the quality, efficacy, and safety of the formulation [7].

XRPD is the gold standard for the physical characterization of API and DDSs. Vibrational spectroscopy is also commonly used for qualitative and quantitative analysis of DDSs. [85,106,140] Vibrational spectroscopy is a powerful tool that facilitates the evaluation of the molecular structure of materials and can distinguish between differences in crystalline forms. They are generally considered fast, accurate, and reproducible experimental methods (i.e., Raman and IR spectroscopy). [10,140] It became evident during the compilation of this review that Raman spectroscopy [5,88] is commonly used to characterize DDSs, most likely due to the noted advantages. The determination of the structural properties is also crucial for developing a DDS and can be determined using a wide variety of methods. This can include; diffraction based techniques and nuclear magnetic resonance (NMR) [141].

In a study on oral delivery of vaccine using microdevices, spray-dried cubosomes with ovalbumin (OVA) and Quil-A (coated using pH-sensitive lid) were characterized using different techniques including, cryo-transmission electron microscope (TEM), dynamic light scattering (DLS), small-angle X-ray scattering (SAXS) (Fig. 5a, b). [142] Cryo-TEM was shown to provide higher resolu-

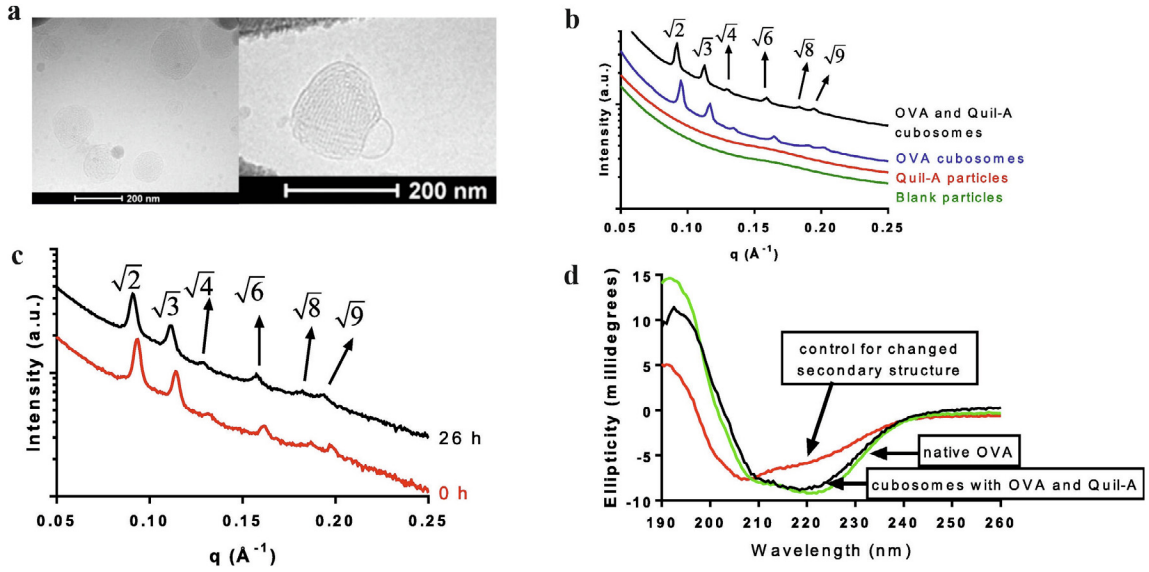

Fig. 5. a) Cryo-TEM images of hydrated cubosomes showing their substructures, **b)** SAXS diffractograms of blank (no Quil-A or ovalbumin), Quil-A particles, ovalbumin (OVA) cubosomes, & OVA and Quil-A cubosomes, **c)** SAXS diffractograms where the morphology of OVA and Quil-A was found it be relatively similar after 26 h of being stored in aqueous dispersion. This is confirmed by the limited changes in the location of the Bragg peaks, **d)** CD spectra of OVA and Quil-A with cubosomes after spray drying, where control for changes secondary structure is red (after OVA boiled for 2 h), native OVA is green, cubosomes with OVA and Quil-A is black [142]. All figures reproduced with permission from Elsevier. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

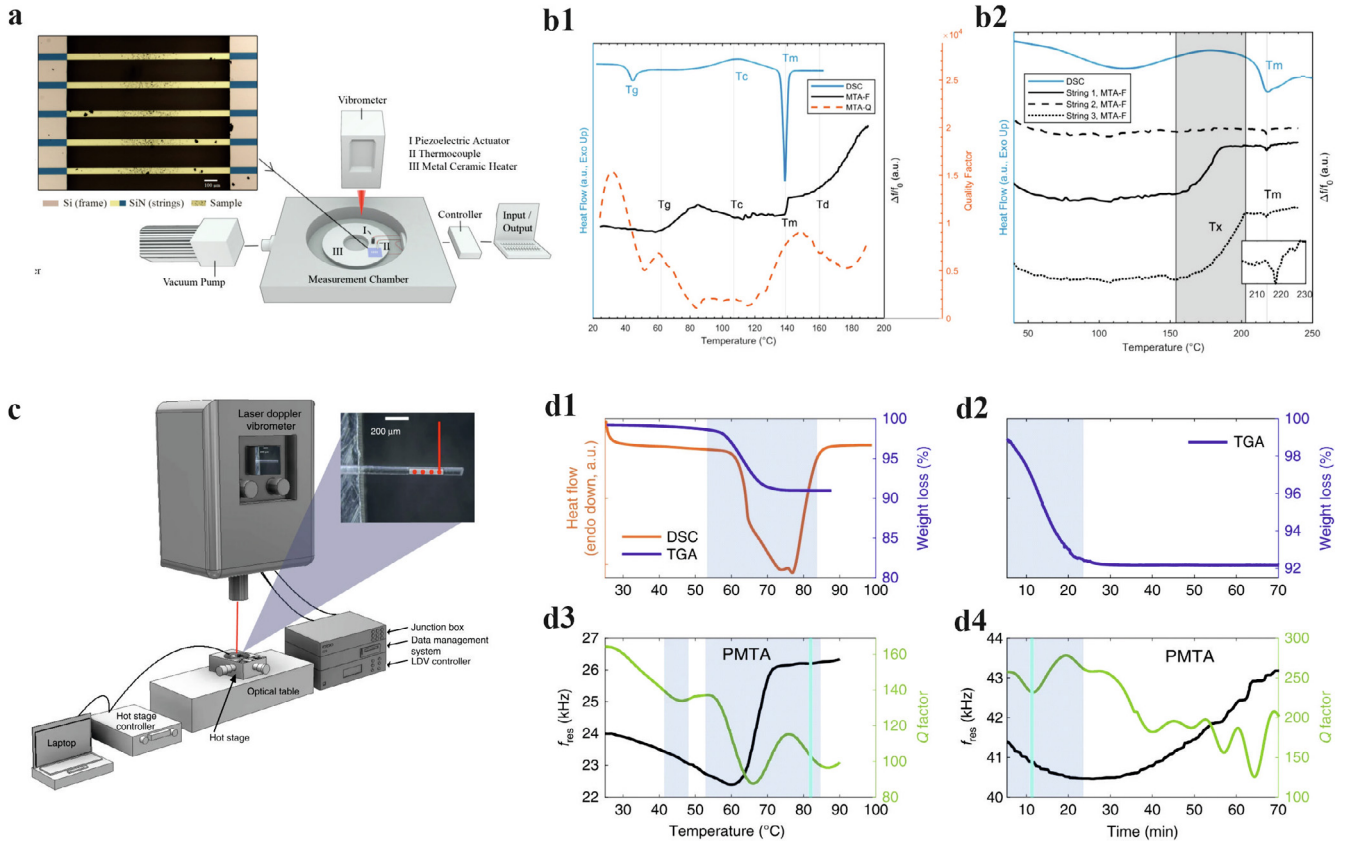

Fig. 6. Novel methods for physical characterization of small and macromolecules. **a)** Experimental setup for micromechanical thermal analysis (MTA) [63], **b1)** comparison between DSC and MTA thermograms of cimetidine at 10 °C/min with MTA showing multiple transitions that are undetected by DSC [63], **b2)** DSC thermogram of a lysozyme (heated at 10 °C/min) with repeatability (three) measurements on three silicon nitride strings, **c)** schematic showing the experimental setup of the PMTA showing a zoom in on a single particle image with a measurement grid (vertical red line is a laser), **d1)** thermograms from a DSC and TGA instrument showing the dehydration of drug called theophylline monohydrate (TP MH) where the grey shaded area is showing the main phase transitions, **d2)** isothermal dehydration of TP MH using TGA 50 °C over 70 min, **d3)** single particle TP MH dehydration from 25 to 90 °C, **d4)** PMTA isothermal dehydration of TP MH at 50 °C [52]. Reproduced with permission from the American Chemical Society and b) – d) is shared under a Creative Commons Attribution 4.0 International License. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tion images compared to SEM, therefore allowing for the visualization of model API (ovalbumin) in the hydrated state. The morphological structure of OVA and Quil-A appeared to be showing some similarities and differences after 26 h (Fig. 5c). As an analytical technique, SAXS is very sensitive to conformational changes (Fig. 5d) occurring in API in solution and provides information about the subtle differences between the different components at a spatial resolution of between 1 and 100 nm. [143] These benefits motivated its use in this study.

4.2. Novel MEMS technologies for small-scale characterization

The mentioned methods in the previous sub-section were mainly bulk methods. however, MEMS technologies are becoming increasingly popular for characterizing the physical stability of API and polymers with even pictogram amounts. [61,62] MEMS resonators have been experimentally tested and shown to be very sensitive in detecting phase transitions in materials. An example is with string resonators used to gain a fundamental understanding of the physical stability of amorphous compounds during heating (Fig. 6a, b1, b2). [63] More recently, it was discovered that the physical stability of API and polymers could be investigated using the material as a resonator – also named particle mechanical, thermal analysis (PMTA) (Fig. 6c, d1, d2, d3, d4). [52]

These different methods offer the possibility of characterizing small amounts of material (pico-nanogram) and being superior in sensitivity compared to bulk techniques, which may be beneficial in early drug development. [52] The main challenge of these novel techniques is their introduction and potential implementation in drug discovery. They are still in the early stages of research, and it is still not entirely clear how small scale characterization can be used to understand better the stability of the API in a formulation (i.e. drug product).

4.3. Spatial confinement of amorphous & crystalline drugs in the drug delivery device

Amorphous compounds (small molecules) are generally more soluble than crystalline forms of APIs; however, their physical stability is of concern because of the tendency of the API to recrystallize from an amorphous to a crystalline solid form. This can happen in varying environmental conditions (i.e., temperature, humidity, or pressure). Fig. 7a1-a4 show microcontainers (empty and) loaded with the API indomethacin in its amorphous form. Nielsen et al. showed that microcontainers could increase the physical stability of amorphous indomethacin in comparison to bulk formulations (Fig. 7b-c) [64]. Applying molecular (Raman) and bulk (XRPD) level characterization methods, it was possible for the authors to investigate the physical stability of indomethacin.

The preparation method of amorphous solid forms can lead to differences in the physical stability of the loaded oral drug delivery devices, as demonstrated using indomethacin (API). [96] In another

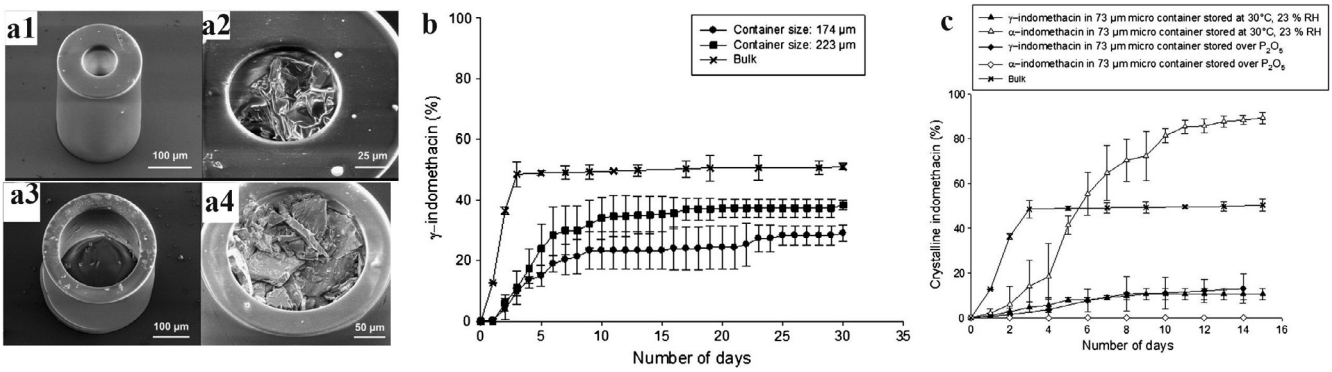

Fig. 7. Investigation of amorphous compounds in microcontainers. SEM images of **a1)** a empty microcontainer (73 ± 5 µm), **a2)** amorphous indomethacin-loaded microcontainer (73 ± 5 µm), **a3)** empty microcontainer (223 µm), **a4)** amorphous indomethacin-loaded microcontainer 223 µm. **b)** Recrystallization of amorphous indomethacin in 174 µm and 223 µm. The samples were stored at 30 °C and 23% RH, **c)** Recrystallization of amorphous indomethacin in 73 µm containers when stored at 30 °C, 23% RH, P_20_5, which reflects the impact of storage on *in vitro* release [64]. All figures reproduced with permission from Elsevier.

study by Nielsen et al. (2014), it was shown that using different cooling rates when preparing the API leads to differences in the physical properties of the API, such as particle size. [144] Confocal Raman microscopy was used to characterize the API, and a standard µ-dissolution profiler was used to determine the release profile of the API. More investigations into the stability of formulations in DDSs are needed.

As previously noted, Raman spectroscopy is commonly used to characterize DDSs. [24,46,145,146] Here, Mazzoni et al. used a custom-built 3D-volumetric Raman imaging setup for monitoring the physical stability of drug-excipient mixtures (Polyvinylpyrrolidone and naproxen), in a microcontainer device (Fig. 8a). XRPD was performed to determine the polymorphic solid form of naproxen, the excipient PVP, and the contents of the loaded formulation in different sized microcontainers (Fig. 8b). A similar set of experiments was permed using confocal Raman microscopy. The main difference is that XRPD measures the averaged response (bulk) of the samples, whereas Raman spectroscopy probes the molecular structure of the samples (Fig. 8c). The authors also show the distribution of naproxen in the individual microcontainers (Fig. 8d). Naproxen exists in different [147] solid forms, which motivated the use of orthogonal techniques in this work [105]. One of the claims of this custom setup is the possibility to image the API encapsulated in the device up to several microns in depth. These studies could also have been performed with diffraction-based techniques in large infrastructures (i.e., synchrotron).

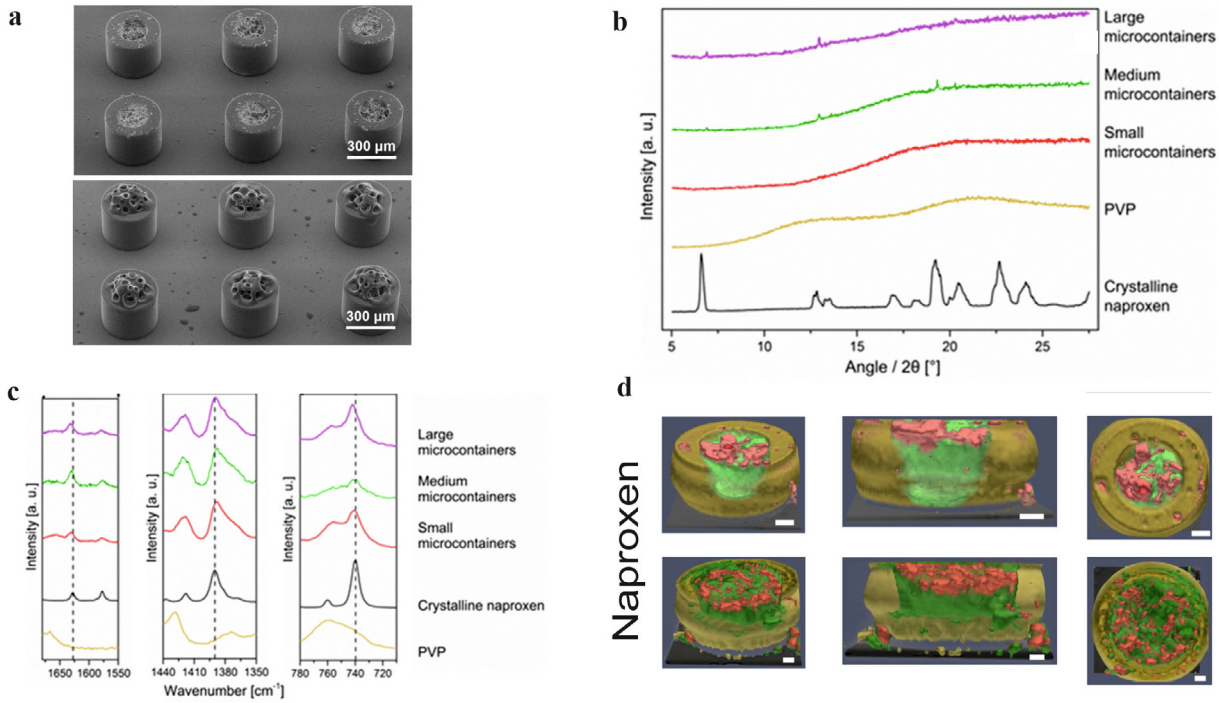

Fig. 8. Physical characterization of Naproxen. a) SEM images of SU-8 microcontainers internal diameter of 97 ± 6, 191 ± 9, and 413 ± 5 µm, respectively, **b)** X-Ray powder diffractograms of crystalline naproxen, polyvinylpyrrolidone (PVP), small, medium and large SU-8 microcontainers that are loaded with PVP then impregnation with naproxen, **c)** Raman spectra of pure crystalline naproxen, naproxen in different sized SU-8 microcontainers (small, medium and large), and PVP, **d)** 3D Volumetric Raman maps of naproxen and PVP in large and medium sized microcontainers with a scale bar (white line) corresponding to 50 µm [105]. Figures reproduced with permission from the American Chemical Society.

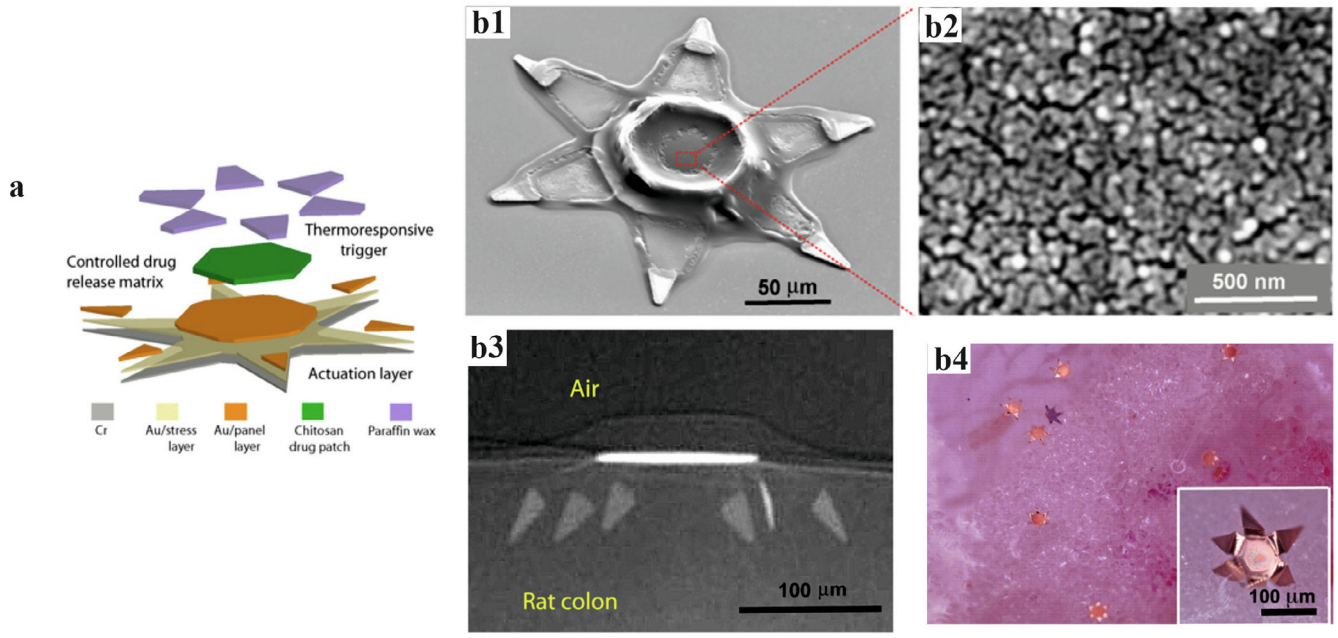

Fig. 9. a) Schematic of the microfabrication of a 250 μm theragripper device. b1) encapsulated with drug at the center (red lines show this), **b2)** API encapsulated chitosan patch on the device, **b3)** ex vivo rat colon μ-CT image of theragrippers inside a rat colon, **b4)** ex vivo image showing theragripper adhering onto the mucosa of the colon (ex vivo), with the inset showing a zoom in on a single theragripper on the colon [37]. All figure shared under a Creative Commons Attribution 4.0 International License. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.4. Visualization of API in the drug delivery systems and in the gut

Visualization and morphology characterization is primarily performed using an SEM and this is well established, as stated in section 2. X-ray micro-computed tomography is a technique that is used to obtain 3D images without destroying the microdevice. [148] Imaging of loaded ODDS in the GI tract is normally attempted using μ-CT, as it gives unique insights into the location of the delivered devices and whether they are performing as expected.

For example, theragrippers (Fig. 9a), a shape changing oral/rectal DDS, were developed by Ghosh et al. for improving the mechanical adhesion in the GI tract. [149] Fig. 9b1shows an SEM image of the 250 μm device. The authors demonstrated the functionality of this ODDS (model compounds chitosan) (Fig. 9b2) in the colon of a rat. The colon was imaged using SEM, μ-CT (spatial resolution of 1.36 μm), and bright field imaging for the *in vivo* and *ex vivo* experiments.[37] Fig. 9b3 shows the cross section of a rat colon *ex vivo* (Fig. 9b4), with the theragripper devices penetrating up to 30–40 μm into the colon. Using μ-CT the authors could determine the depth of penetration of the theragripper DDS into the colon.

However, μ-CT does not give any structural information that could be used to determine the structural changes occurring during the penetration process. More recently, Kjeldsen et al. used X-ray imaging and computed tomography scanning to visualize and track 300 μm diameter microdevices *in vivo*. This approach enabled quantitative evaluation of the retention of oral delivery devices in the GI track on rats. [150].

4.5. Example of a DDS stability study

Only a limited number of physical and chemical stability studies have been performed with ODDSs. This may be due to the size, complexity, and solubility of these devices. A recent example by Caffarel-Salvador et al. demonstrated the importance of conducting stability studies. The authors developed a novel buccal delivery platform that could deliver high drug loads (2 mg of API on a 10 mm x10 mm patch) of human insulin and human growth hormone in the buccal area. [151] Due to this relatively high payload and the intended clinical trials of this ODDS, stability studies were performed.

PVP and sorbitol were evaluated as binders in the formulation (API-loaded microneedle device), and their stability was investigated at different environmental conditions, 5 °C, 25 °C, and 40 °C (and 75% RH) at time 0, 1, 2 and 3 months of storage. After storage at the noted conditions, the API was filtered for quantification using HPLC. The findings suggested that different binders (PVP and sorbitol) have minor stability differences after 3 months of storage at 5 °C for 3 months, due to the presence of covalent dimers. There were also no significant changes in the formulation when stored at 40 °C.

In general, the excipients that are added to a drug delivery device can influence the physical and chemical stability of the ODDS, and this needs to be well understood by performing environmentally controlled experiments. [49,50,81]. It is worth noting that this study is unique because it presents findings ranging from *in vivo* to clinical studies, in phase one, with 100 human volunteers. Stability studies are required for the transition from drug discovery to clinical studies.

5. *In vitro* systems for the evaluation of release, adsorption, and efficacy

The success rate of conventional DPs depends on predicting the efficacy and toxicity of the drug in the preclinical stage. Likewise, ODDSs must undergo rigorous screening studies in the discovery and developmental phases, as highlighted in the sections above. The release of API from the delivery device is commonly evaluated in *in vitro* drug dissolution studies. Adsorption studies typically carried out using artificial membranes or cell and tissue layers. The drug absorption studies across the intestinal barrier [128] could be studied using artificial membranes, octanol-water partition, *in silico*, *in situ perfusion*, *in vivo*, and *in vitro* models (cell or tissue-based).[152-154] Among these models, *in vitro* models have been widely used, not just due to the ethical and financial consid-

eration but also because of their compatible results in human subjects and animals. [155,156]

The utility of any *in vitro* platform lies in its ability to have well-controlled test conditions for studying ODDS performance. [157] The success rate of using *in vitro* model ultimately depends on how well they can mimic conditions of *in vivo* intestinal epithelium. Hitherto, developing an ultimate *in vitro* system simulating all the conditions in the human intestine is rather complex. Therefore, *in vitro* platforms characterize some specific aspects of ODDS where the conditions are easier controlled than *in vivo*. [156,158] In the following sub-sections, we will focus on the relevance and use of various *in vitro* platforms to study ODDS's performance.

5.1. In vitro models

In vitro dissolution models are used to evaluate drug efficacy and to study the kinetics of drug release. [159] The ODDSs are often designed with the goal of reaching targeted, controlled, and prolonged release of drugs instead of burst release for improved therapeutic effect. [160] Monitoring the drug release from ODDSs in liquid media (e.g., simulated intestinal fluid) is usually measured principles based on fluorescence and UV absorbance. [55,66,161,162] For modulating the release characteristics of API from delivery devices, the devices can be encapsulated in biodegradable polymers (Fig. 3a1-a3) to tune the dissolution and release kinetics of drugs as previously discussed in section 3. [101] For example, PLGA is a material that is used for the fabrication of degradable carrier with the goal of controlled release of oral peptides. [163,164]

There are now several polymers available, including commercial polymers such as Eudragit® extensively used for various encapsulation strategies, [118,161,165,166] thus increasing the demand for rapid characterization of coatings to improve the design iteration and fabrication cycles. The inherent advantage of *in vitro* dissolution models provides comparatively faster alternatives before performing cell-based studies or *in vivo* experiments. However, due to the use of several manual handling steps and the need for using large reagent and sample volumes, the use of *in vitro* dissolution models poses several practical challenges. As a potential solution, *in vitro* experimental models could possibly be miniaturized and automated. To this end, Rajendran and co-workers have proposed a real-time microfluidics-based approach for quantifying the drug release from ODD based on electrochemical detection. [111,159]. In this study, the ODDs were placed in a sample loading chamber along with the carrier buffer, and the buffer flow was based on centrifugal force. This method not only increases automation, significantly lowers the needed sample volume but also has the possibility for multiplexing for *in vitro* testing.

5.2. Caco-2 Transwell® cultures

Commonly, oral absorption studies are carried out using a cell-monolayer seeded on top of a polycarbonate membrane in a Transwell® culture plate (Fig. 10a). [167,168] A widely used cell line for permeability, transport, and adsorption studies is the Caco-2 cell line. Caco-2 cells are human epithelial cells from colon adenocarcinoma and are used as a model of the intestinal barrier. [152] After long-term culture in confluent monolayers (cells in tissue layer), these cells differentiate into intestinal enterocyte-like cells, with developed features such as apical brush borders, polarization, and tight junctions. [169] The barrier integrity and the strength of the tight junctions are estimated by measuring the *trans*-epithelial electrical resistance (TEER) before testing with ODDS. Although Transwell® cell cultures are predominantly used for drug transport studies, their use for the characterization of ODDS is limited and scarce in the literature [108-110,116].

Levy and co-workers [108] studied the effect of hydrogel DDSs for reversibly inhibiting the efflux transporters in the GI epithelial cells. They investigated the drug absorption and toxicity of the hydrogel microdevice. Levy et al. seeded Caco-2 cells on high-density polycarbonate Transwell® inserts and cultured the cells for 21 days, after which they performed viability studies using stains and visualized via a confocal microscope (Fig. 10b1-b2). To increase the complexity of the Caco-2 cell monolayer, they added a biosimilar mucus to the apical chamber. One of the interesting observations from this experiment was the effect of the microdevice on the inhibition of P-gp (an efflux inhibitor) that took at least 4 h, compared to the microdevice performance without the biosimilar mucus. This delayed effect is due to the mucous barrier affecting the mobility of microdevices and the permeation of small molecules. This implies that the barrier complexity of an *in vitro* platform significantly affects the characterization aspects of ODDS.

In efforts to improve the cellular complexity of the intestinal epithelium models, HT29 cells and Raji B cells could be co-cultured along with the conventional Caco-2 cell model. HT29 cells help in mimicking the mucus-secreting Goblet cells, while Raji B cells play the role of inducing the Caco-2 cells to gain an absorptive phenotype resembling M cells. [173] Nevertheless, the use of such co-culture models for characterizing ODDS is still elusive due to various practical concerns such as the complexity of setting up a co-culture as well as the handling of drug delivery devices in a culture plate, even in a Caco-2 monoculture [109].

5.3. Perfusion systems

Although Transwell® cell culture models enable molecular transport studies across the transepithelial barrier, these models are still static. These cell culture models do not mimic the intraluminal flow, which is a characteristic feature of the intestinal microenvironment *in vivo*. Moreover, the complex structure of the intestinal barrier, such as the villi structures, is not replicated in these models. Such systems fail to mimic the microenvironment encountered by the ODDS and it's associated *in vivo* biological complexity.

In order to replicate the shear stress encountered by ODDS in the intestinal microenvironment, Fisher and co-workers implemented a mucin flow system over a Caco-2 monolayer [116]. Their study investigated the impact of GI retention of ODDS based on mucoadhesive agents such as lectin compared to the geometry of the ODDS (beads vs. nanowires). Unmodified control-beads and lectin-modified beads were easily detached at the lowest shear rate of 2.35 and 3.6 dynes/cm^2, respectively. Unexpectedly in their study, the lectin-modified nanowires, which were expected to have higher retention, showed lower shear stress survival at 5.7 dynes/cm^2 compared to unmodified nanowires at 9.15 dynes/cm^2. The authors detail that lectins could bind to both the cells and mucus, leading to competitive binding, thus reducing mucoadhesion. This is one of the limitations of using a mucin flow system when the mucoadhesive agent interacts with both the cells and the mucin. However, there are few studies available that successfully demonstrate the mucoadhesive property of ODDS using mucin flow. [36,110,116]

Microfluidic based perfused cell culture systems have been extensively used for absorption, distribution, metabolism, and excretion (ADME) testing. [174,175] Particularly, the concept of gut-on-a-chip to mimic the intricacies of the small intestine *in vitro* has become an excellent tool for studying gut physiology (Fig. 10c1-c4). The current application scope and future opportunities of the gut-on-a-chip system have been reviewed extensively elsewhere. [176] Despite the versatility of microfluidic platforms, when aiming for testing ODDS (100–500 μm or few mm), there is a requirement for experimental platforms that are comparatively

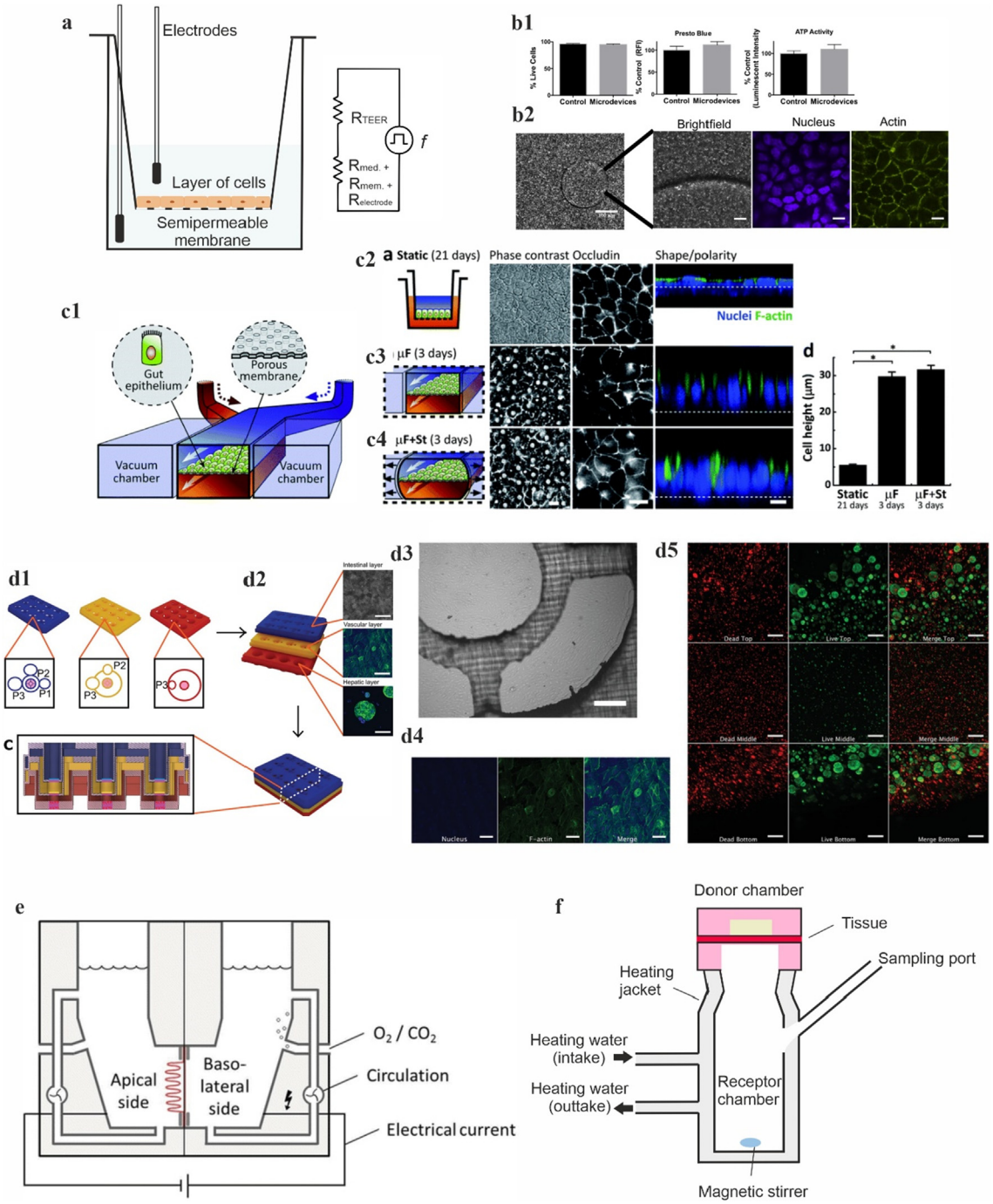

Fig. 10. ***In vitro*** **systems for the evaluation of release, adsorption, and efficacy of ODDs. a)** Schematic representation of static Transwell® culture setup. Caco-2 cells are cultured as monolayer over a permeable membrane, separating the apical (top) and basolateral compartments. The inset shows the TEER electrical diagram. The electrical resistance of the total system includes the ohmic resistance of the cell layer (R_{TEER}), permeable membrane ($R_{Mem.}$), cell culture medium (T_{Med}), and electrode medium interface ($R_{Electrode}$), **b1)** biocompatibility studies of microdevices on Caco-2 cells using fluorescence stain propidium iodide stain (dead), protease blue assay, and ATP assay, **b2)** confocal images of a microdevice (scale bar = 100 μm) incubated for 180 min over Caco-2 cells grown on Transwell for three weeks displaying microdevices in brightfield, DAPI nucleus stain (blue), phalloidin actin (green). This is a representative image of n = 3 (scale bar = 10 μm) [108], **c1)** schematic representation of a gut-on-a-chip microfluidic device with a porous membrane covered by gut epithelial cells horizontally across the central microchannel. Morphology difference of Caco-2 cells cultured in Transwell® system vs, **c2)** gut-on-a-chip with microfluidic flow, **c3)** without and **c4)** with cyclic mechanical strain, system layout schematics (left); distribution of the tight junction protein, occludin (center) in the epithelial monolayers, vertical cross section of epithelium depicting cell shape and polarity (right) (nuclei in blue and F-actin in green; scale bar, 20 μm), **c5)** average height of Caco-2 cells grown in static Transwell cultures, in the microfluidic gut-on-a-chip without or with mechanical strain [170], **d1)** cartoon representation of the top view of 3D printed tissue-model inserts with Caco-2 cells in blue, HUVEC cells in yellow, and HepG2 cells in red. Hydrogels are depicted in pink color. The sampling holes are placed at each level: P1, P2, and P3 for sampling blue, yellow and red inserts, respectively, **d2)** schematics of assembled tissue inserts (scale bar 100 μm), x) with a side view of the assembled tissue-model inserts, **d3)** bright-field microscopy image of HUVEC cells on the growth matric after seeding (scale bar 100 μm), **d4)** F-actin (green) and nucleus (blue) stain of HUVEC cells (scale bar 50 μm), stain of HepG2 cells cultured for 20 days, **d5)** the live (green) and dead (red) distribution in the top, middle and bottom of the hydrogel (scale bar 200 μm) [171]; Representative image of **e)** Ussing chamber [172] and, **f)** Franz diffusion cell. Figures b) – d) reproduced with permission from respective publishers and figure e) shared under a Creative Commons Attribution. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

larger than traditional microfluidic channels, which are generally in the 100 μm range. [176]

Recently, Jepsen and co-workers proposed a unique 3D printed stackable titer plate insert to support three interconnected tissue models (intestine, liver, and blood vessel) for drug transport studies. [171] The study aimed at designing and testing easily fabricated 12-well plate stackable inserts (Fig. 10d1) to address the limitations of Transwell® and microfluidics for *in vitro* cell models. Their triple-layered design is based on commercially available inserts for cell culture plates. These three layers are designed such that the cell layers could be independently cultured (due to different time and medium for development) and swiftly assembled before usage (Fig. 10d2). The ultimate advantage of this experimental platform is that the smallest well of this presented system was 5 mm, enabling ODDS (100–500 μm) to be characterized using this platform. Additionally, Birk et al. recently showed the advantages of an *in vitro* cell-based centrifugal microfluidic platform to evaluate the efficacy of functionalized antibiotic carrying microdevices on biofilm [177].

Despite the accepted accuracy of simple cell-line models for mechanistic studies, the lack of feedback mechanism of numerous interconnecting and cross talking cell types in the *in vitro* cell culture models are still disadvantageous. [178] For translating and extrapolating *in vitro* data to the *in vivo* conditions, the used model should reasonably reflect the complexity of the test microenvironment. For this aspect, tissues *ex vivo* provides a good alternative with functions of various cell types still preserved. [157]

5.4. Ex vivo tissue models

In vitro tissue-based models can also be termed *ex vivo* models, involving experiments with functional living tissues or organs isolated from an animal and maintained outside the living organism in a highly controlled condition. [157] These experimental models are significantly different from the cell-based models in terms of better paracellular permeability supplied by the intestinal epithelium, mucus layer, expression of transport proteins, and drug metabolism [153,155,179]. A more thorough comparison between cell-based models and tissue-based models was reviewed by Nunes et al. previously [180]. The following sub-sections are primarily focused on experimental platforms for intestinal drug permeation and accurate prediction of intestinal absorption.

5.5. Diffusion chambers: Ussing chamber and Franz cell

Drug permeability studies are performed using diffusion chambers to predict drug permeation and absorption in the human intestine accurately. Depending on the flux direction of the solution, diffusion chambers could be termed as horizontal (e.g., Ussing chamber) or vertical (e.g., Franz cells). Ussing chamber is the most utilized diffusion chamber for drug permeability studies, while the usage of Franz cells is increasing. [180]

Hoyer et al. used an Ussing-type chamber to determine the unidirectional release of model compounds from their GI patch as wells for permeation studies using rat intestine. [103] The tested GI patch had a mucoadhesive front and non-adhesive back side. To differentiate the drug release from the mucoadhesive front and the back side, the patch was affixed in an Ussing-type chamber. The system (Fig. 10e) consisted of two chambers placed next to each other with an opening. The test patch was placed on the apical side of the intestinal mucosa in between these two chambers and filled with transport buffer solution. The amount of model API released was quantified using a plate reader after collecting samples from both chambers. Likewise, the permeation of the API in the GI patch was studied using an Ussing-type chamber. In this case, however, the sampling was drawn from the acceptor compartment of the chamber and subsequently measured using UV absorbance. It is important to note that these permeation experiments were performed in a controlled atmosphere of 95% O_2 and 4% CO_2 at 37 °C, right after retrieving the excised intestines from a sacrificed rat. This atmosphere of O_2 and CO_2 ensures sufficient tissue oxygenation and also ensures fluid circulation in both compartments.

Another type of diffusion chamber is the Franz cell. [181,182] However, the Franz cells for intestinal permeation studies are less used and are primarily implemented for *ex vivo* skin permeation studies. [183] Franz cell (Fig. 10f) operates on a similar principle as the Ussing chamber and the primary technical difference is that the donor compartment is filled with lower volume in comparison with the receptor compartment.

Jørgensen and co-workers used a Franz cell to study the permeation of insulin from microcontainers developed for oral drug delivery of insulin. [112] After tissue preparation and immobilization in the Franz cell, the donor compartment was filled with 1.5 mL FD70 solution (hDMEM base) while the receptor compartment was filled with 7 mL of hDMEM [hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) - buffered Dulbecco's Modified Eagle's Medium (DMEM)]. The permeation experiment is initiated by positioning microcontainers over the intestinal mucosa with the reservoir opening of the devices facing towards the mucosa. After periodic sampling from the reservoir compartment, the permeated insulin is quantified using an Enzyme-linked immunosorbent assay.

The authors' reason for using Franz cell over Ussing chamber was due to the simplicity of Franz cell for studying unidirectional release over a horizontal barrier. Furthermore, in their case, controlling the orientation of microdevices towards the mucosa in a vertical barrier was a practical challenge. However, they could have benefitted from using an Ussing chamber to enable prolonged viability of tissue using Krebs-Ringer bicarbonate buffer with oxygenation (O_2: CO_2). [156] Another aspect this study points out is the storage conditions of tissue samples. Previously, studies have demonstrated the viability of tissue samples between frozen-thawed colorectal tissues and freshly excised samples. Among them, freezing of tissues leads to poor tissue integrity. [183] Therefore, drug permeation studies are affected, and tissue preparation also becomes crucial for a successful characterization while using *ex vivo* platforms. [180]. For ensuring reproducibility and reliability of data from *ex vivo* tissue models, several parameters such as choice of incubation buffers, assessing tissue viability and integrity using appropriate techniques, along with tissue preparation need to be standardized.

6. Future perspectives and conclusion

Due to the need for innovative DDSs to tackle the delivery of drugs orally, new μm and mm ODDSs are emerging. It is evident that there is a need for cross-disciplinary research environments for their development and characterization. We foresee that new ODDSs will have an important role to play in the delivery of small and macromolecules in therapeutic indications that require repeated dosing over prolonged periods. This could motivate players in the field of new ODDSs since these systems have the potential to facilitate, e.g., targeted, sustained, and triggered release to improve local delivery and to reduce the frequency of dosing in patients. Global leaders such as the World Health Organization are keen to engage on the topic of adherence to medicines. [184]

However, looking ahead, several challenges need to be addressed. To realistically move into clinical testing that eventually benefits patients and society, ODDSs will need to comply with strict regulations on testing and manufacturing from a device

and DP perspective [185]. This is probably also why we only found a limited number of reported clinical studies [78,151]. There are multiple reasons for this, including (but not limited to) lack of sufficient information in the methods used, pressure to publish, inadequate experimental controls or reporting experimental conditions, lack of standardized techniques for the characterization and evaluation of these devices and DDSs, variations in suppliers and tendency to primarily publish positive results [186]. The ODDS research field may be facing issues with reproducibility from lab to a lab that hinders validation, which needs to be addressed [187].

For clinical studies and future translation to the public domain, there is an increasing need to understand better the effect and behavior of ODDSs in the GI tract. Additionally, there should be an increased focus on; the stability of API during processing and during storage in the ODDS, interaction with and impact on body tissue, and material compatibility of drug delivery devices and formulations. A template that may be used is with LBDDS that may provide an example of the needed characterization steps for ODDSs (some) to reach clinical development [16,60,94,188-190]. The use of computational approaches may also facilitate the translation of these systems and should be further investigated [14,191,192].

Moreover, since ODDS can vary significantly in design and intended use, some sensing technologies and experimental platforms may need to be rethought and revaluated. The inventive nature of the ODDS has so far called for the development of a variety of custom-made characterization systems that eventually need to be standardized. Since the ODDS, to a large degree, are emerging out of the field of micro and nanofabrication, we believe that many of the new tools for characterization will likewise take inspiration from micro and nanotechnology research. For example, mucoadhesion of single devices can be measured using a setup inspired by atomic force microscopy. Physiochemical and mechanical characterization can also be performed on extremely small (picogram) amounts of drugs in great detail using nanomechanical resonators. Raman based technologies seem promising to investigate API stability, as it is a non-destructive method where controlled experimental conditions could be performed.

In the context of miniaturized technologies, microfluidics might be used for quality control of ODDSs, since they facilitate *in vitro* studies as well as the handling of individual ODDSs [111]. Due to their general low output per analysis, multichannel microfluidic devices are being developed [193].

For *in vivo* studies, new strategies for bioimaging are needed. Many of the emerging ODDSs involve reconfigurable devices and/or μm - mm sized individual devices that are being developed, need to consider orthogonal techniques during characterization of the API/polymers as well as the DDS. There will be a future need to follow individual devices and the release of cargo over time until they are ingested during *in vivo* and clinical studies. Additionally, high temporal resolution imaging of DDSs to understand/optimize, e.g., DDS working principles and mitigate potential adverse effects, would be invaluable.

In conclusion, the ongoing development of new ODDSs will demand the establishment of new characterization and testing systems – probably a combination of modified standard analysis techniques and entirely new methods emerging from the fields of especially optics and mirco/nano technology. A crucial challenge will be to find characterization methods that are compatible with pharmaceutical manufacturing and quality control processes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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