Collecting e-cigarette aerosols for in vitro applications: A survey of the biomedical literature and opportunities to increase the value of submerged cell culture-based assessments

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Abstract
Electronic nicotine delivery systems (ENDS) are being developed as potentially reduced-risk alternatives to the continued use of combustible tobacco products. Because of the widespread uptake of ENDS—in particular, e-cigarettes—the biological effects, including the toxic potential, of their aerosols are under investigation. Preclinically, collection of such aerosols is a prerequisite for testing in submerged cell culture-based in vitro assays; however, despite the growth in this research area, there is no apparent standardized collection method for this application. To this end, through an Institute for in vitro Sciences, Inc. workshop initiative, we surveyed the biomedical literature catalogued in PubMed® to map the types of methods hitherto used and reported publicly. From the 47 relevant publications retrieved, we identified seven distinct collection methods. Bubble-through (with aqueous solvents) and Cambridge filter pad (CFP) (with polar solvents) collection were the most frequently cited methods (57% and 18%, respectively), while the five others (CFP + bubble-through; condensation; cotton filters; settle-upon; settle-upon + dry) were cited less often (2–10%). Critically, the collected aerosol fractions were generally found to be only minimally characterized chemically, if at all. Furthermore, there was large heterogeneity among other experimental parameters (e.g., vaping regimen). Consequently, we recommend that more comprehensive research be conducted to identify the method(s) that produce the fraction(s) most representative of the native aerosol. We also endorse standardization of the aerosol generation process. These should be regarded as opportunities for increasing the value of in vitro assessments in relation to predicting effects on human health.

KEYWORDS
aerosol collection, e-cigarette, in vitro assays, literature survey, submerged cell cultures

1 | INTRODUCTION

Electronic nicotine delivery systems (ENDS) are being developed as potentially reduced-risk alternatives to the continued use of combustible tobacco products (Brandon et al., 2015; Farsalinos & Polosa, 2014). Electronic cigarettes (e-cigarettes)—one of the most widely known ENDS—consist of a battery-powered device that heats an “e-liquid” contained inside an atomizer, which leads to generation...
of an inhalable aerosol upon puffing by the user (McRobbie, Bullen, Hartmann-Boice, & Hajek, 2014). E-cigarette devices can vary extensively in terms of design and functionality (Breland et al., 2017). Similarly, e-liquids are also highly diverse and can contain different levels of nicotine, flavoring agents, and humectants such as propylene glycol and vegetable glycerin (Brown & Cheng, 2014).

The recent rise in the use of e-cigarettes around the world has brought the toxicity of their aerosols into focus (Callahan-Lyon, 2014; Orr, 2014). Many institutes, including those from industry, academia, and government, are conducting research to understand their toxicological hazard and risk potential. As in other areas, much of the preclinical research on e-cigarette-derived aerosols is performed in vitro cell culture models because they are relatively inexpensive (compared with animals), amenable to different types of higher-throughput analyses, supportive of the 3Rs principles (to Replace, Reduce, and Refine animal usage in scientific experiments), and importantly, the data generated from these models are potentially translatable to higher levels of biological organization. Significantly, in vitro toxicology data can influence the development of an e-cigarette device or e-liquid formulation. However, because many in vitro assays are conducted in submerged two-dimensional cell cultures, the aerosol generated from an e-cigarette must first be collected before it can be applied to the cell model under investigation. This challenge was originally faced by researchers seeking to investigate the toxicity of cigarette-derived smoke in vitro (Bradford, Harlan, & Hamner, 1936). Ultimately, relatively standardized processes were developed whereby the smoke from combustible tobacco products was generated via smoking machines and subsequently collected in several ways (reviewed in Klus, Boenke-Nimphius, & Müller, 2016), including (a) total particulate matter or condensate captured on a Cambridge (glass fiber) filter pad (CFP) and desorbed with dimethyl sulfoxide (DMSO); (b) condensate captured via electrostatic precipitation (EP) and solubilized in DMSO; (c) condensate captured in a cold trap; and (d) aqueous solution (AQ)-soluble gas-vapor phase (GVP) constituents captured in phosphate-buffered saline (PBS). Some of these collection methods can be applied in tandem—for example, sequential CFP- or EP- and AQ-mediated trapping—and they can produce fractions that are broadly representative of the composition of tobacco smoke when considered as a whole (Klus, Boenke-Nimphius, & Müller, 2016). Although parallels can be drawn between cigarettes and e-cigarettes in the context of smoke and aerosol collection for in vitro applications, the latter products are more contemporary than the former and, consequently, have not been subjected to the same degree of experimentation. Thus, the general level of knowledge that has been built over the decades in relation to smoke generation and collection, at present, exists only minimally for e-cigarette-derived aerosols. Hence, there might be scope to ameliorate various aspects of the procedures linked to e-cigarettes.

The Institute for in vitro Sciences, Inc. (IVS) is currently hosting a series of workshops that provide a forum for stakeholders to identify, discuss, and develop recommendations for optimal generation of test samples and use of genetic toxicology in vitro assays to support tobacco product regulatory requirements (Moore et al., 2020). This workshop series follows two previous IVS workshops that focused on in vitro models for chronic obstructive pulmonary disease and in vitro exposure systems and related dosimetry (Behnsing et al., 2016; Behnsing et al., 2017). Because evaluation of ENDS represents a new challenge for in vitro testing, practical issues associated with these products are a major focus of the current IVS workshop series. During the initial workshop, the participants agreed on the need for reviewing the state of the science in relation to e-cigarette aerosol collection for in vitro applications, and, predicated on this consensus view, a survey of the biomedical literature was conducted in order to map the types of methods employed for this purpose. The present publication provides a summary of this survey. It should be noted that in vitro systems composed of cells cultured at the air-liquid interface coupled with whole aerosol exposure technologies were out of scope for this survey because it is a highly specialized area of research and merits its own dedicated review. Importantly, the opportunities arising from this survey should be exploited to improve the understanding and study of e-cigarette-derived aerosols in submerged cell culture-based in vitro assays.

2 | METHODOLOGY

2.1 | Literature search

We conducted a search via PubMed® (https://www.ncbi.nlm.nih.gov/pubmed)—the freely accessible literature repository containing >30 million publications from the fields of biomedicine and health (PubMed, 2020)—to identify all potentially relevant publications for subsequent evaluation. The most recent search was conducted during December 2019. We used the following search terms: (“electronic cigarette” AND “All Fields”) OR “electronic cigarettes” AND “All Fields”) OR “e-cigarette” AND “All Fields”) OR ((“electronic nicotine delivery systems” AND “MeSH Terms”) OR ((“electronic” AND “All Fields”) AND “nicotine” AND “All Fields”) AND “delivery” AND “All Fields”) AND “systems” AND “All Fields”) OR “electronic nicotine delivery systems” AND “All Fields”) OR “e-cigarettes” AND “All Fields”). Results were filtered by year (2013–2019: the time period when the vast majority of these publications was published) and reviews were excluded. Publications were further triaged by evaluating their abstracts (exported from PubMed®) for the following keywords: Aerosol; Capture; Collection; Condensate; Emissions; Immobiliz(s)ation; In vitro; Oxidative; Toxicity; Toxicology; Trapping; Vapo(u)r.

2.2 | Data extraction, compilation, and visualization

Triaged publications were critically evaluated for the presence of empirical information related to collection of e-cigarette aerosols for evaluation in vitro assays. Relevant data were subsequently extracted and used to compile a database; the categories of data extracted are described in Tables 1 and 2. Note that, in publications that assessed more than one “item,” the items were grouped together for entry into the database. For instance, a publication that evaluated 39 e-liquids and 5 e-cigarette devices via two different vaping
regimens would be represented in the relevant fields of the database as "39 Types," "5 Types," and "2 Types," respectively. In addition, database fields were completed as 'not available' (N/A), where relevant information was lacking or not explicit. Data were visualized by using Spotfire® Desktop (v7.13.0, TIBCO®, Palo Alto, CA, USA).

3 | RESULTS

3.1 | General database statistics

The initial search retrieved 4561 publications. From these, further keyword triaging identified 1543 publications. Upon inspection, 47 publications from the 1543 were found to contain relevant empirical data, while the remaining were rejected because of lack of direct relevance to e-cigarette aerosol collection for in vitro application and/or absence of empirical information. Interestingly, two of these publications reported two distinct collection methods each (Breheny et al., 2017; Rayner et al., 2019). Thus, in total, there were 49 individual collection methods itemized in the database. Selected database statistics are described in Table 1.

3.2 | Collection method-related information

Table 2 provides a summary of the relevant data. Seven distinct collection methods were reported in the 47 publications; these were defined as "bubble-through," "CFP," "CFP + bubble-through," "condensation," "cotton filters," "settle-upon," and "settle-upon + dry." Each collection method is described in the Section 4, while graphical illustrations are presented in another publication emanating from the IVS workshop series (Wieczorek et al., 2020). Bubble-through and CFP were the most frequently cited collection methods (57% and 18%, respectively), while the others were cited less often (2–10%) (Figure 1).

In addition, eight different solvent systems were used in these collection methods, including "25% DMSO/Water/PBS," "DMSO," "DMSO + PBS," "fetal bovine serum (FBS)," (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES)-buffered saline," "medium," "methanol (MtOH)/DMSO," and "PBS" (Table 2). Note that four studies applied the ‘condensation’ collection method without a solvent and, thus, were represented with N/A in these fields of the database. Consequently, on the basis of the collection methods and solvents employed in the 47 publications, we defined seven different categories of aerosol fraction(s): "AQ-soluble aerosol collected matter (ACM)," "AQ-soluble condensate," "aqueous extract (AQE)," "condensate," "DMSO-soluble ACM," "DMSO-soluble ACM + AQ-soluble GVP," and "MtOH-/DMSO-soluble extract" (Table 2). A graphical summary of this information is provided in Figure 2.

3.3 | Additional findings

In general, there was large heterogeneity among the other e-cigarette aerosol generation-related parameters in the 47 publications. For example, the collected aerosols were generated from a multitude of e-cigarette devices and e-liquids via numerous different commercially available smoking machines or laboratory-built apparatuses (Figure 3). Furthermore, there was also diversity in the vaping regimens and number of puffs applied for aerosol generation (Figure 4). In addition, a number of studies performed very limited chemical characterization of the collected e-cigarette aerosols (i.e., nicotine quantification) (Table 2).

4 | DISCUSSION

Like in other areas of applied research, in vitro data have an important role to play in helping us comprehend the toxic potential of e-cigarette-derived aerosols in humans, while also supporting the 3Rs principles of scientific animal experimentation. Such data can help not only more readily identify hazards in an animal-cognizant manner but also potentially delineate modes-of-action (Ramirez et al., 2018; Shukla, Huang, Austin, & Xia, 2010); ultimately, this information will add to the weight-of-evidence that informs the risk assessment of e-cigarettes in relation to human health. Although sophisticated approaches involving three-dimensional organotypic respiratory tract cell cultures and whole aerosol exposure systems that partially recapitulate physiologically relevant exposure in humans might eventually become the key model for studying aerosol-associated toxicity, they are, at present, in their infancy and still require further exploration and validation (Bishop et al., 2019; Czekala et al., 2019; Iskandar et al., 2019; Mathis et al., 2013). Oustensibly until then, submerged two-dimensional cell culture-based assays will represent the core of in vitro assessments, in particular, for the internationally accepted tests that are used for identifying genotoxic and cytotoxic hazards, such as the in vitro micronucleus, mouse lymphoma, bacterial mutagenicity, and neutral red uptake assays (INVITTOX, 1990; OECD, 1997; OECD, 2016a, 2016b). However, for the in vitro data to hold appreciable value in this context, it is vital that the captured aerosol is representative of its native aerosol. Significantly, underpinning this requirement is the collection method and solvent(s) employed. To this

| Parameter     | Details                                      |
|---------------|----------------------------------------------|
| Publications  | 47                                           |
| Individual    | 49                                           |
| collection    | methods                                      |
| Primary       | ≥34                                          |
| institutes     |                                              |
| Publication    | 2013–2019                                    |
| range         |                                              |
| Research areas| Immunology; inflammation; oral health;       |
|               | oxidative stress; tissue repair; toxicology; |
|               | vascular                                     |

**Table 1** Selected database statistics


### TABLE 2  Summary of the collection method-related information from the 47 publications and additional data

| Collection method | Manuscript reference | PMID | Collection solvent(s) | Fraction(s) | E-cigarette device | E-liquid | Smoking machine | Puffs | Vaping regimen | Nicotine quantified |
|-------------------|----------------------|------|-----------------------|-------------|-------------------|----------|----------------|-------|----------------|---------------------|
| Bubble-through    | Breheny, Oke, Pant, & Gaça, 2017 | 28 444 993 | Medium | AQE | Vype ePen | Blended Tobacco | SM-450 | 10 | CRM No. 81 | Yes |
|                   | Rayner, Makena, Prasad, & Comet-Boyaka, 2019 | 31 166 129 | Medium | AQE | Innokin VV4 / Nautilus tank Tobacco | N/A | N/A | 55-mL vol, 5-s draw, 30-s interval | Yes |
| Munakata et al., 2018 | 30 227 175 | Medium | AQE | 2 types | N/A | N/A | 300 | HCI | Yes |
| Taylor et al., 2017 | 28 658 606 | Medium | AQE | 2 types | N/A | Blended tobacco | RM20H | 10 | CRM No. 81 | Yes |
| Bengalli, Ferri, Labra, & Mantecca, 2017 | 29 053 606 | Medium | AQE | Kit / Simple Ribilio / C14 Passthrough | 12 types | TRUST-iCERT | 200 | 55-mL vol, 3-s draw, 60-s interval | No |
| Behar et al., 2016 | 27 633 763 | Medium | AQE | 2 types | 39 types | N/A | 24 | 2 types | No |
| Farsalinos et al., 2013 | 24 135 821 | Medium | AQE | 2 types | 21 types | Vacuum | N/A | 2 types | No |
| Ganapathy et al., 2017 | 28 542 301 | HEPES-buffered saline | AQE | 2 types | 5 types | Vacuum | N/A | HCI | Yes |
| Rubenstein, Hom, Ghebrehiwet, & Yin, 2015 | 26 072 673 | HEPES-buffered saline | AQE | 2 types | 3 types | Vacuum | N/A | N/A | No |
| Ji et al., 2016 | 28 033 425 | Medium | AQE | N/A | 4 types | Homemade | N/A | 33- to 83-mL vol, 2- to 5-s duration | No |
| Anderson, Majeste, Hanus, & Wang, 2016 | 27 613 717 | Medium | AQE | 4 types | Tobacco | Vacuum | N/A | 55-mL vol, 2-s duration, 30-s interval | No |
| Teasdale, Newby, Timpson, Munafò, & White, 2016 | 27 137 404 | Medium | AQE | Aerotank Mini / iStick battery | Haven fluid USA Mix | N/A | 5 | 5.8-mL vol, 5-s draw, 10-s interval | Yes |
| Taylor et al., 2016 | 27 690 198 | Medium | AQE | 2 types | Blended tobacco | RM20H | 10 | CRM No. 81 | Yes |
| Collection method | Manuscript reference | PMID | Collection solvent(s) | Fraction(s) | E-cigarette device | E-liquid | Smoking machine | Puffs | Vaping regimen | Nicotine quantified |
|-------------------|----------------------|------|-----------------------|-------------|--------------------|---------|----------------|-------|----------------|-------------------|
| Omaiye, McWhirter, Luo, Pankow, & Talbot, 2019 | 30 896 936 | Medium | AQE | 8 types | Peristaltic pump | N/A | 43- to 56-mL vol, 4.3-s draw, 60-s interval | Yes |
| Leslie et al., 2017 | 28 470 141 | Medium | AQE | 15 types | Diaphragm pump | 14 | ISO | No |
| Rankin et al., 2019 | 30 957 912 | Medium | AQE | 2 types | Water aspirator | 13 | 1.5-s draw, 30-s interval | No |
| Kaisar, Sivandzade, Bhlerao, & Cucullo, 2018 | 29 879 439 | PBS | AQE | N/A | SCSM | 8 | FTC | No |
| Bharadwaj, Mitchell, Qureshi, & Niazi, 2017 | 27 875 752 | Medium | AQE | Classic tobacco | Vacuum | N/A | N/A | Yes |
| Yu et al., 2015 | 26 547 127 | Medium | AQE | 2 types | Vacuum | N/A | N/A | No |
| Di Blase, Attoni, Di Benedetto, & Sanchez, 2018 | 30 575 566 | FBS | AQE | 2 types | Vacuum | N/A | 10-s draw | No |
| Higham et al., 2016 | 27 184 092 | Medium | AQE | 6 types | Peristaltic pump | N/A | 2 types | No |
| Higham, Bostock, Booth, Dungwa, & Singh, 2018 | 29 615 835 | Medium | AQE | VS/CES clearomiser/VIP battery | USA tobacco | Peristaltic pump | N/A | N/A | No |
| Hom et al., 2016 | 27 096 416 | HEPES-buffered saline | AQE | 5 types | Vacuum | N/A | N/A | No |
| Otręba, Kosmider, Knyżak, Warncke, & Sobczak, 2018 | 29 665 082 | Medium | AQE | eGo-3 twist battery/bottom headed clearomizer | Palaczbot | 6 | 70-mL vol, 1.8-s draw, 17-s interval | No |
| Ravez-Villanueva, Ma, Kleiboer, & Holloway, 2018 | 30 048 688 | Medium | AQE | EVOD Kangaroo-Techn | N/A | N/A | N/A | Yes |
| Ween, Whitall, Hamon, Reynolds, & Hodge, 2017 | 28 867 672 | Medium | AQE | EVOD-2 | N/A | 10 | 3-s draw, 5-s interval | No |
| Zahedi et al., 2019 | 31 200 115 | Medium | AQE | 2 types | UoK ASM | N/A | 4.3-s draw, 60-s interval | No |
| Zhao et al., 2018 | 29 102 637 | Medium | AQE | 2 types | ECAG | N/A | 2 types | Yes |
| Collection method | Manuscript reference | PMID | Collection solvent(s) | Fraction(s) | E-cigarette device | E-liquid | Smoking machine | Puffs | Vaping regimen | Nicotine quantified |
|--------------------|----------------------|------|-----------------------|-------------|--------------------|----------|-----------------|-------|----------------|---------------------|
| CFP                | Breheny, Oke, Pant, & Gaça, 2017 | 28 444 993 | DMSO | DMSO-sol ACM | Vype ePen | Blended tobacco | LM20X | 40 | CRM No. 81 | Yes |
|                   | Rayner, Makena, Prasad, & Comet-Boyaka, 2019 | 31 166 129 | Medium | AQ-sol ACM | Innokin VV4/Nautilus tank | Tobacco row | N/A | N/A | 55-mL vol, 5-s draw, 30-s interval | Yes |
|                   | Thorne et al., 2016 | 27 908 385 | DMSO | DMSO-sol ACM | Vype ePen | Blended tobacco | LM20X | N/A | CRM No. 81 | No |
|                   | Misra, Leverette, Cooper, Bennett, & Brown, 2014 | 25 361 047 | PBS | AQ-sol ACM | blu eCigs | 4 types | Vitrocell VC10 | N/A | HCl | Yes |
|                   | Husari et al., 2016 | 26 272 212 | Medium | AQ-sol ACM | V4L CoolCart/Vapor Titan Soft Touch battery | Strawberry | ONARES | N/A | 80-mL vol, 4-s duration, 14-s interval | No |
|                   | Shaito et al., 2017 | 29 079 789 | Medium | AQ-sol ACM | V4L CoolCart | Strawberry | ONARES | N/A | 80-mL vol, 4-s duration, 14-s interval | No |
|                   | Daleympile et al., 2018 | 30 346 667 | DMSO | DMSO-sol ACM | NVP | Twilight tobacco | LM20X | 200 | CRM No. 81 | No |
|                   | Ito et al., 2019 | 31 400 404 | DMSO | DMSO-sol ACM | Vype ePen | Blended tobacco | RM20D | N/A | CRM No. 81 | Yes |
|                   | Thorne et al., 2019 | 31 163 219 | DMSO | DMSO-sol ACM | N/A | N/A | RM200a | 60 | CRM No. 81 | Yes |
| CFP + bubble-through | Takahashi et al., 2018 | 29 158 044 | DMSO + PBS | DMSO-sol ACM + AQ-sol GVP | NTV | N/A | Rotary | 70 | HCl | No |
| Condensation       | Scott et al., 2018 | 30 104 262 | N/A | Conden-sate | Kanger | 2 types | N/A | N/A | 3-s draw, 30-s interval | Yes |
|                    | Clapp et al., 2017 | 28 495 856 | N/A | Conden-sate | LAVABOX DNA 200/SOK TFV4/TF-CLP2 Clapton coil | 7 types | Peristaltic pump | 20 | 30-s Interval | No |
|                    | Lei, Lerner, Sundar, & Rahman, 2017 | 28 256 533 | N/A | Conden-sate | eGO/eGo Vision Spinner battery | 4 types | Peristaltic pump | N/A | 4-s draw, 30-s interval | No |
|                    | Schweitzer et al., 2015 | 25 979 079 | N/A | Conden-sate | Innokin iClear 16 | 3 types | Vacuum | N/A | N/A | No |
|                    | Sun, Kosinska, & Guttenplan, 2019 | 31 373 329 | 25% DMSO/Water/PBS | AQ-sol condensate | 2 types | 2 types | ASPECG pump | N/A | ISO | Yes |
| Collection method | Manuscript reference | PMID | Collection solvent(s) | Fraction(s) | E-cigarette device | E-liquid | Smoking machine | Puffs | Vaping regimen | Nicotine quantified |
|--------------------|----------------------|------|----------------------|-------------|--------------------|----------|----------------|------|----------------|---------------------|
| **Cotton filters** | Miyashita et al., 2018 | 29 437 942 | PBS | AQ-sol ACM | RBC CES Clearo-mizer | 2 types | Peristaltic pump | 25 | N/A | No |
| **Settle-upon** | Shivalingappa, Hole, Westphal, & Vij, 2016 | 26 377 848 | Medium | AQE | Kangar EVOD | Flavorless | Motor | N/A | N/A | No |
| | Behar, Wang, & Talbot, 2018 | 28 596 276 | Medium | AQE | iClear16D dual coil/ Innokin iTaste MVP 3.0 battery | 45 types | Peristaltic pump | N/A | 56-mL vol, 4.3-s draw, 60-s interval | No |
| | Alani, Park, Chakir, Semlali, & Rouabhia, 2018 | 29 800 583 | Medium | AQE | Smooth Canadian tobacco | Peristaltic pump | N/A | 10-s draw, 30-s interval | No |
| | Zahedi, Phandthong, Chaii, Remark, & Talbot, 2018 | 30 032 837 | Medium | AQE | N/A | 2 types | N/A | N/A | 4.3-s draw, 60-s interval | No |
| **Settle-upon + dry** | Tommasi, Bates, Behar, Talbot, & Besaratinia, 2017 | 29 191 599 | MtOH/ DMSO | MtOH/ DMSO-sol extract | 3 types | N/A | Peristaltic pump | 10 | 3 types | No |

Abbreviations: ACM, aerosol collected matter; ASPECG, aerosol single port electronic cigarette generator; AQ, aqueous solution; AQE, aqueous extract; CRM, CORESTA recommended method number 81, CORESTA recommended method number 81 (square wave puff profile, 55-mL vol, 2-s draw, 28-s interval); DMSO, dimethylsulfoxide; ECAG, e-cigarette aerosol generator; FTC, Federal Trade Commission (bell-shaped puff profile, 35-mL vol, 2-s draw, 58-s interval); FBS, fetal bovine serum; GVP, gas-vapor phase; HCl, Health Canada Intensive (bell-shaped puff profile, 55-mL vol, 2-s draw, 28-s interval); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ISO, International Organization for Standardization 3308, (bell-shaped puff profile, 35-mL vol, 2-s draw, 58-s interval); FBS, fetal bovine serum; GVP, gas-vapor phase; HCl, Health Canada Intensive (bell-shaped puff profile, 55-mL vol, 2-s draw, 28-s interval); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ISO, International Organization for Standardization 3308, (bell-shaped puff profile, 35-mL vol, 2-s draw, 58-s interval); MtOH, methanol; N/A, not available; NTV, novel tobacco vapor product; NVP, novel vapor product; ONARES, oro-nasal respiratory exposure system; PBS, phosphate-buffered saline; SCSM, single cigarette smoking machine; s, seconds; sol, soluble; vol, volume; UoK ASM, University of Kentucky analytical smoking machine.
end, we surveyed the biomedical literature from the PubMed® repository for the approaches used by different laboratories across the world in their published in vitro research on collected e-cigarette-derived aerosols.

Among the 47 relevant publications identified in the survey (including studies Breheny et al., 2017 and Rayner, Makena, Prasad, & Cormet-Boyaka, 2019 that described two methods each), 57% (28/49) of the collection methods were defined as "bubble-through." This is a method whereby aerosol generated from an e-cigarette is bubbled into a solvent, resulting in a solution containing the aerosol constituents, which can be subsequently applied to cell cultures. Furthermore, all 28 examples found in this survey employed water-based protic solvents—cell culture medium, FBS, PBS, or HEPES-buffered saline—and, thus, generated AQEs. It is, therefore, hypothesized that water-soluble constituents are captured predominately via this collection method, while other constituents—poorly and nonwater-soluble compounds for example—are probably not. Crucially, it should be noted that there is a disturbing lack of chemical characterization data on the collected aerosols among the studies in general; this is a critical finding, which we will address later in the manuscript. Nevertheless, there is empirical support for the theory of water-soluble constituent trapping via bubble-through/aqueous solvent-related methods from analytical studies on cigarette-derived GVP collected in PBS. These reports indicate that chemicals such as carbonyls, including acids, esters, amides, imides, aldehydes, and ketones, as well as lactones, alcohols, pyridine derivatives, imidazoles, lactams, and nitrogen

### Figure 1
Types of methods employed for collection of e-cigarette-derived aerosols for in vitro research. CFP, Cambridge filter pad.

### Figure 2
Solvents used in collection of e-cigarette aerosols and, consequently, the fraction(s) evaluated in in vitro research. AQ, aqueous solution; AQE, aqueous extract; ACM, aerosol collected matter; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; GVP, gas–vapor phase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MeOH, methanol; N/A, not available; PBS, phosphate-buffered saline; sol, soluble.
FIGURE 3  E-liquids, smoking machines, and e-cigarette devices (annotated within the figure) used in generation of aerosol(s) for in vitro research. ASPECG, aerosol single-port electronic cigarette generator; N/A, not available; NTV, novel tobacco vapor product; NVP, novel vapor product; ONARES, oro-nasal respiratory exposure system; SCSM, single cigarette smoking machine; UoK ASM, University of Kentucky analytical smoking machine.

FIGURE 4  Number of puffs and vaping regimens used in the generation of aerosol(s) that were collected for in vitro research. CRM No. 81, CORESTA recommended method number 81; FTC, Federal Trade Commission; HCI, Health Canada Intensive; ISO, International Organization for Standardization 3308; N/A, not available; s, seconds; vol, volume.
heterocyclic compounds can be collected effectively by the bubble-through method (Noya et al., 2013; Schumacher, Green, Best, & Newell, 1977). In contrast, it is also recognized that numerous harmful and potentially harmful constituents from cigarette smoke (e.g., benz[a]pyrene, dibenzo[a,h]pyrene, and 5-methylchrysene) are highly lipophilic (i.e., possessing octanol–water partition coefficients [log P] > 5) (Smith & Hansch, 2000). Thus, if these types of molecules are present in the aerosols produced from e-cigarettes, they are most likely not captured by aqueous solvent-centric methods because of their inherent chemical properties.

The next most frequently employed collection method was CFP (18%; 9/49). In this method, e-cigarette-derived aerosol is pulled through a CFP to capture its constituents on the filter pad (i.e., ACM). These constituents are subsequently desorbed and solubilized in a solvent. These nine publications employed different polar solvents, both protic (cell culture medium and PBS) and aprotic (DMSO) in nature, which yielded fractions that were defined as AQ- or DMSO-soluble ACM. Importantly, total particulate matter fractionated from cigarette smoke has been extensively characterized owing to the virtues of CFP-mediated collection (Chepiga et al., 2000; Roemer et al., 2004). Thus, while the same kind of particulate matter (carbon-based) is not present in the aerosol of e-cigarettes because of the absence of combustion (Lampos et al., 2019), one might expect that the approach has the potential to capture a similar profile of chemicals, although the adherence capacity of aerosol components towards the CFP as well as their solubility in the applied solvent will obviously dictate which constituents finally comprise the fraction. Predicated upon published examples, this approach can potentially trap chemicals such as nicotine, glycerol, aromatic amines, and polycyclic aromatic hydrocarbons (Chepiga et al., 2000; Roemer et al., 2004). Interestingly, a recent publication reported that a CFP method is more effective in collecting a targeted set of flavor chemicals than its bubble-through counterpart, indicating that this sorbent-based technique might have advantages over others (Eddingsaas et al., 2018). However, one possible limitation of this capture method is that aerosol constituents not retained by the CFP are, presumably, poorly collected.

The tandem combination of CFP and bubble-through methodologies (defined as "CFP + bubble-through") was cited once in this selection of curated literature (2%; 1/49). DMSO was used to solubilize and elute ACM from the CFP, while PBS was used to capture a portion of the constituents passing through the CFP; thus, it is anticipated that the two fractions contained polar/nonpolar and non-CFP immobilized water-soluble constituents, respectively. When aerosol is collected in this manner, toxicological assessment of both fractions—as was done in previous in vitro assessments of cigarettes and heated tobacco products (Gonzalez-Suarez et al., 2016; Rickert, Trivedi, Momin, Wright, & Lauterbach, 2007; Roemer et al., 2015; Schaller et al., 2016)—might provide a better understanding of the hazard potential of the aerosol in its entirety.

The "condensation" collection method was cited five times (10%; 5/49), and, in four of the five studies, fractions defined as condensates were produced and then assessed in vitro. While this approach has potential advantages (e.g., circumventing the need for a collection sorbent or solvent), it is not clear which, and at what proportion, aerosol constituents (other than nicotine) are condensed and, therefore, present in the final fraction. In the fifth study, the condensate was subsequently solubilized in a solvent system comprising 25% DMSO, water, and PBS (Sun, Kosinska, & Guttenplan, 2019). However, similar to its related fractions, the composition of this particular fraction (AQ-soluble condensate) is also not apparent.

In addition, three other aerosol collection methods were described among the selected publications. The method defined as "settle-upon" was cited four times (8%; 4/49). Essentially, in this method, e-cigarette aerosol is allowed to settle upon the solvent in question (cell culture medium in these specific cases), and, presumably, the aerosol constituents are eventually taken up by and solubilized in it. A similar collection method, namely, "settle-upon + dry," was applied once (2%; 1/49). Here, following aerosol settling and solubilization, the original solvent (MIOH) is removed by drying, and the resultant residue is resolubilized in a second solvent (DMSO). The efficiency of these methods in collecting aerosol constituents is also unknown; however, the drying step of the latter method might cause the loss of any volatile compounds and inadvertently prevent their presence in the ultimate fraction. The final collection method, "cotton filters," was also cited only once (2%; 1/49). In essence, this method replicates the CFP method, although it provides a different and potentially less efficient sorbent (owing to varying pore sizes) than the glass fiber material of the CFP. Moreover, in this case, a water-based protic solvent (PBS) was used to solubilize the captured ACM; thus, it is likely that the final fraction mainly contained water-soluble constituents adhered to the cotton filters.

Aerosol collection methods (including solvents) were not the only parameter to vary among the 47 publications. There was also large heterogeneity among other key study elements, including the e-cigarette devices, e-liquids, smoking machines, puffs taken, and vaping regimens (as exemplified in Figures 3 and 4). Thus, while it is the prerogative of the institute undertaking the research to employ the materials and experimental conditions that meet its needs, it might be beneficial to promote the use of certain standards, such as topography research-based vaping regimens, in order to standardize common aspects of aerosol generation, as was done for cigarettes in the past (CMR, 2018; Health Canada, 1999; ISO, 2012). Published recommendations from the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) could also be leveraged in this regard (CORESTA, 2015).

More notably, however, as described above, the publications evaluated as part of this survey reported very limited chemical characterization data on the collected aerosols, if any at all. We assume that this type of analysis was generally not performed; however, it is theoretically possible that the data have just not been reported. Nevertheless, nicotine was quantified in several of the publications despite the use of different collection methods, indicating that at least this one prominent aerosol constituent was present in the various fractions produced (i.e., AQS, AQ- and DMSO-soluble ACM, and condensate). Interestingly, in addition to performing aerosol collection via the bubble-through methodology (into cell culture medium) for assessing
cytotoxic potential, one publication also reported analogous activities—but by using isopropanol as the solvent instead—in order to investigate in parallel the transfer of flavor chemicals from the e-liquid to the captured aerosol (Omaie, McWhirter, Luo, Pankow, & Talbot, 2019). Although the AQE applied to the cell cultures in this case was not the subject of characterization, the use of a potentially more effective trapping solvent (isopropanol rather than cell culture medium) still resulted in relatively poor collection (>50% transfer efficiency) of this chemical family subtype. These findings suggest that the AQEs generated via the bubble-through methodology might not accurately represent the native aerosol, at least in terms of flavor content. Linked to these results is a broader point in relation to flavors. Given their volatile nature, no collection method might be truly optimal to capture all flavor chemicals, although further research (as described below) is required to inform this discussion.

In light of the findings of this survey, our fundamental concern relates to the apparent dearth of information on the overall chemical characteristics of the collected e-cigarette aerosols. Without knowing the chemical composition of the fraction(s) evaluated, it is not possible to draw strong conclusions on the associated in vitro data. Thus, in order to enhance the utility of such data in this context, we recommend that research efforts be focused on chemically characterizing the fractions generated by each type of collection method in a comprehensive fashion. This work should endeavor to identify the collection method(s) and solvent(s) that produce the fraction(s) most representative of the native aerosol that is amenable to evaluation in submerged cell culture-based assays. Furthermore, the chemical stability of the collected aerosol fractions should also be studied in order to ascertain their shelf life and optimal storage conditions, given that samples might be transported and stored before use. Establishing these conditions would pave the way for greater standardization of the entire e-cigarette aerosol collection process for in vitro applications and, critically, raise the value of existing and future in vitro data on this topic. Interestingly, two recent publications described non-targeted screening methodologies (based on gas chromatography with time-of-flight mass spectrometry) that were used to characterize the trapped emissions of tobacco products and e-cigarettes (Knorr et al., 2019; Rawlinson, Martin, Froxina, & Wright, 2017). We envisage that similar approaches could be employed in the research proposed here in order to uncover in greater detail the chemical composition of the various aerosol fractions.

5 | CONCLUDING REMARKS

In the present PubMed® survey, we identified seven types of methods that were used to collect the aerosol from e-cigarettes for assessment in submerged cell culture-based in vitro assays. The different collection methods (and associated solvent systems) have been appraised here to some extent by using the limited analytical data reported in the 47 publications themselves as well as supporting data generated elsewhere; however, we call for more comprehensive research into the chemical nature of the fractions produced in order to enhance our knowledge of their composition. In addition, we also endorse greater levels of standardization (in aerosol generation parameters, for example) in order to increase the levels of consistency among testing laboratories. Exploiting these opportunities would serve two purposes, both of which ultimately aim to support our comprehension of the effects of e-cigarette-derived aerosols on human health: (a) to improve our collective understanding of the in vitro assay data already reported in the public domain and (b) to ensure that the most effectual data are generated from in vitro studies in the future.

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