Acquisition of aseptic techniques using virtual reality: a randomized trial on performance, emotion and experience

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Abstract

The aseptic technique is essential for minimizing contamination in cell and tissue culture. However, biomedical science undergraduates in Universiti Putra Malaysia face challenges in acquiring hands-on learning experience on proper aseptic techniques due to inadequate facilities, which decreases learning interest and efficiency. To address this, an in-house virtual reality (VR) mobile application, AsepticTech VR, was developed to simulate a cell and tissue culture facility for practicing aseptic techniques. This study aims to investigate the efficacy of AsepticTech VR as a teaching and learning tool. Forty-one biomedical undergraduates were randomly assigned into test and control groups. Both groups attended a pre-test to evaluate the pre-intervention learning performances. Then, only test group participants were given access to AsepticTech VR, while both groups attended traditional lectures and practical sessions for a week. After one week, all participants took a post-test to evaluate their post-intervention learning performances. Additionally, test group participants also completed the modified Differential Emotions Scale (mDES) questionnaire to assess their emotions associated with using AsepticTech VR, and Web-Based Learning Tool (WBLT) questionnaire to assess their learning experience. The results showed that the test group participants had better learning performance improvements in the cognitive (p<0.05) and psychomotor domain (p<0.01) compared to the control group but did not differ in their affective domain. Moreover, the test group demonstrated stronger positive emotions compared to negative emotions (p<0.001) and had positive learning experience with AsepticTech VR. This indicates that AsepticTech VR is an effective teaching and learning tool for cell and tissue culture aseptic techniques.

Keywords: Virtual reality; aseptic techniques; learning performance; learning emotion; learning experience; cell and tissue culture

1.0 Introduction

Aseptic technique refers to a set of protocols that aims to minimize contamination. It is an umbrella term that encompasses the creation of a sterile field for work to the delicate arrangement and careful maneuver of items during the work. The aseptic technique is essential to biomedical research that heavily relies on cell and tissue culture as *in vitro* research models. The presence of contaminants in cultures may disrupt their physiology and growth, thereby affecting the physiology and confounding experimental results (Nikfarjam & Farzaneh, 2012; Phelan & May, 2015). Besides, the contamination of culture with human pathogens is a potential source of laboratory-associated infections that pose a significant risk to laboratory operators and personnel (Uphoff et al., 2010). As such, it is evident that the acquisition of proper knowledge and training on the topic of aseptic technique is crucial in cell and tissue culture, therefore sufficient exposure and training should be provided to involved personnels. Furthermore, contamination in cell and tissue culture also leads to loss of resources, as contaminated cells and reagents are usually discarded. In essence, aseptic techniques are central to the effectiveness of cell and tissue culture work.

Given the importance of aseptic techniques, training courses on these skills are frequently included in the undergraduate curriculum of bioscience undergraduate studies (Wang et al., 2018). However, despite their value, such modules are typically not incorporated in a manner that enables proper hands-on training in an actual cell and tissue culture facility. Proper implementation of such modules is impaired by the limited access to a fully-functional and well-maintained cell and tissue culture facility, such as a biosafety cabinet (BSC). Besides, the sheer amount of material, human, and time resources required for a truly rigorous training programme on aseptic technique is perceived to have a low return-of-investment (Bowey-Dellinger et al., 2017). As such, students are often met with the disappointment of resorting to traditional lecture sessions that expose students to theoretical knowledge or aseptic techniques, or open-bench task simulation sessions that aim to familiarise students with the skills and movements. Consequently, this disappointment could significantly reduce students' interest and motivation to learn about this crucial topic in biomedical research, which may negatively impact learning efficiency (Erickson et al., 2020).

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In light of the limitations, our team devised an in-house mobile virtual reality (VR) application named AsepticTech VR that simulates a cell and tissue culture facility for students to gain hands-on learning experience on aseptic techniques. The idea of using VR to provide a learning opportunity through simulation was supported by the multiple benefits VR-assisted learning has on the learning outcomes in various fields. Besides, the use of mobile-platform VR technology is deemed a cost-effective alternative as compared to the establishment of an actual cell and tissue culture facility (Venkatesan et al., 2021). Despite these, there remains a lack of direct, empirical evidence that demonstrates the beneficial impact of VR-assisted tissue culture training (Reen et al., 2021). As such, this study sought to investigate the effectiveness of AsepticTech VR as a teaching and learning tool on the topic of aseptic techniques in cell and tissue culture. We hypothesize that the use of AsepticTech VR will positively impact the learning outcomes.

This study is guided by three main specific objectives. Firstly, given the previously established effectiveness of VR in laboratory education-based teaching and learning activities, we sought to investigate whether the use of AsepticTech VR can enhance the learning performances with regards to cell and tissue culture aseptic techniques. The learning performance outcomes were measured based on the three learning domains via the use of self-developed questionnaires and rubrics (Hoque, 2016). It is hypothesized that AsepticTech VR, when combined with traditional lectures and practical sessions, can significantly improve the users' cognitive, affective, and psychomotor learning performance on aseptic techniques.

Secondly, a vast amount of literature supports the role of emotions in learning and memory (Tyng et al., 2017). Of note, positive emotions are strong predictors of academic performance among learners, while the converse is true for negative emotions (Pekrun et al., 2017). In the specific case of VR-assisted learning, VR technology was shown to significantly enhance positive learning emotions as compared to other methods of learning, which was also associated with increased learning ability (Allcoat & von Mühlenen, 2018). Therefore, it would be interesting to see whether AsepticTech VR is able to elicit similar responses among the students. To this end, the emotions associated with the use of AsepticTech VR were measured via Modified Differential Emotions Scale (mDES) questionnaire (Fredrickson, 2013). AsepticTech VR is hypothesized to induce stronger positive emotions as compared to negative emotions among users.

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Thirdly, the learners' appraisal of a learning application in terms of learning, design, and engagement was shown to be significantly correlated and predictive of learning performances, especially in higher-level learning processes such as analysis and application (Kay & Knaack, 2009). Therefore, investigating students' learning experiences associated with the learning, design, and engagement factors of AsepticTech VR could potentially reveal insights on whether any observed learning performance increases may be associated with increased students' appraisal towards the application. To accomplish this, the learning experience associated with the use of AsepticTech VR was measured via a Web-Based Learning Tool (WBLT) questionnaire (Kay, 2011). AsepticTech VR is expected to provide a positive learning experience to users.

2.0 Literature Review

2.1 Aseptic Techniques in Cell and Tissue Culture

The establishment of a reliable cell or tissue culture research model relies on three essential elements: (1) Integrity, the compatibility of the selected system with its intended use, (2) Authenticity, the identity of the system in terms of phenotype and genotype, and (3) purity, the extent to which a cell and tissue culture system is free from physical, chemical, and biological contamination (Kaplan & Hukku, 1998). With regards to purity, it is therefore important to protect a cell and tissue culture system from contaminants as the presence of contaminants may confound experimental results by altering the culture's physiology or even identity, leading to impaired validity of findings (Buehring et al., 2004; Nikfarjam & Farzaneh, 2012). While certain additives like antibiotics and antifungals may be added to the culture media, it is generally not encouraged to do so as these additives are associated with cytotoxic or cytostatic effect (Lanbeck & Paulsen, 1995; Lanbeck & Paulsen, 2001). Therefore, in a cell and tissue culture laboratory, contaminations are usually prevented rather than handled after their occurrence. Such aim is accomplished via adherence to aseptic techniques.

The aseptic technique refers to a set of principles and protocols that is adhered to minimize the risk of contamination by foreign organisms or cells. Aseptic technique is an umbrella term that encompasses a few major areas, including the establishment of a sterile work area, maintenance of sterile work surface, good personal hygiene, maintenance of sterile reagents and media, and sterile handling of cultures and reagents (Freshney, 2010). In other words, aseptic techniques represent a combination of engineering controls over the environment and personnel control over the handling of consumables. As such, proper aseptic techniques are typically practiced in an established and fully furnished cell-and-tissue culture facility that includes equipment like BSC (Coté, 1998).

Awareness towards the importance of sterile and authentic culture and products are on the rise. For instance, more researchers are now calling for checks on cell-line authenticity that may be altered with cross-contamination between cell lines (Nims & Reid, 2017). Good manufacturing practice (GMP) laboratories also routinely perform quality checks for presence of contamination to detect microbial contamination (Akel, 2015). Nevertheless, according to

the Guidance Document on Good Cell and Tissue Culture Practice (GCCP) 2.0, there is current a lack of early career basic training for laboratory personnels in cell and tissue culture, which increases the risk for contamination during tissue culture work (Pamies, 2021).

Considering its importance, aseptic techniques are often integrated into curriculum plans of biomedical science courses (Wang et al., 2018). Traditionally, classroom lectures are held to expose students to the theoretical aspects of aseptic techniques, but whether such an approach alone suffice to ensure competency of the students in cell and tissue culture aseptic techniques is a matter that remains to be elucidated. However, given that learning of cell and tissue culture aseptic technique relies substantially on hands-on learning experience (Durukan et al., 2020), training of aseptic techniques may alternatively be conducted through practical sessions where students are trained to manually perform the techniques in an actual cell and tissue culture facility (Aruscavage, 2013). To this end, resources in terms of equipment, space, and time often serve as a limiting factor for the mass implementation of hands-on training sessions in an undergraduate classroom (Bowey-Dellinger et al., 2017). While performing aseptic techniques on an open-bench remains an option, the risk for contamination from open-bench work is significantly higher as compared to performing aseptic techniques under proper facilities setting, such as in a BSC (Spencer & Savage, 1975). Therefore, alternative solutions that may replace the need to establish expensive facilities while providing the experience of working in a real cell-and-tissue-culture facility setting should be considered.

Fortunately, the use of virtual reality may be able to address these problems, as the biggest motivation for using virtual reality lies in its ability in providing learners with the opportunity to experience a situation that is usually inaccessible physically (Durukan et al., 2020; Freina & Ott, 2015).

2.2 Virtual Reality in Biomedical Education

Virtual reality (VR) is technological systems that are defined by three principal features: immersion, presence, and interactivity (Mütterlein, 2018). In general, VR is a system with computer-generated three-dimensional (3D) models that enable users to immerse and interact with the virtual environment in a seemingly realistic manner (Abich et al., 2021).

VR usage has become increasingly popular in various fields for training and education purposes, including in medicine, surgery, industry, and cell biology (Venkatesan et al., 2021). In biomedical education, VR has seen several uses and applications. For instance, several studies have employed 3D-visualisation technology including VR in teaching and learning sessions on human anatomy (Yammine & Violato, 2015). Besides, VR technology has been used to visualize 3D models of the internal content of cells to understand cellular architecture and distribution of macromolecules (Stefani et al., 2018). Another study utilized the technology for the teaching and learning of cell biology in sophomore-level classes (Bennett & Saunders, 2019). Beyond academic institutions, VR has been proven effective in the transfer of procedural knowledge, such as standard operational procedures (SOPs) within pharmaceutical industries as compared to learning about the procedures through reading (Wismer et al., 2021).

Overall, the use of VR is associated with beneficial outcomes. A recent meta-analysis by Kim & Kim (2023) found that VR-applied medical education could improve skills and satisfaction levels among healthcare personnel. Another scoping review by Dhar et al. (2023) agreed with the results, revealing several benefits associated with VR-applied medical education, including attainment of knowledge, increase in learning efficacy, increase in confidence, and an overall improvement in self-efficacy with regards to the skills that are taught. In particular, Kwon (2019) has highlighted that experiential learning, which is a form of learning that occurs through direct experiences, is possible via the use of VR and may provide an explanation for the increased learning effect observed. Furthermore, the use of VR also enables students to undergo learning and training sessions that are not bounded by spatial and temporal limitations (Alqahtani et al., 2017; Smutny et al., 2019). Importantly, VR provides the opportunity to practice a skill-of-interest without the real-world consequences of failing (Abich et al., 2021; Tüysüz, 2010).

2.3 Virtual Reality in Cell and Tissue Culture Education

With regards to the specific application of VR in the education of cell and tissue culture aseptic techniques, our literature search revealed a scarcity of these types of studies.

However, three independent studies reported the use of virtual simulation technology in the context of laboratory aseptic technique-related education. The first study, by Kaltsidis et al. (2021), utilized a VR module by the Centers for Disease Control and Prevention (CDC) that

exposes users to the procedures of preparing a class II BSC. The study investigated the learners' experience associated with the use of the application and performed a qualitative review of the learners' perception and appraisal towards the application. A second study, by Yap et al. (2021), adopted the learning module for cell culture basic techniques from Labster in their blended learning activities. In their study, the perception and experience towards the module and their implication in the attainment of learning outcomes were assessed from the perspective of the learners. The final and most recent study by Hemme et al. (2023) employs the use of a self-developed VR application to provide hands-on training of cell and tissue culture methods and aseptic techniques, though no assessments regarding the outcome or appraisal of this application has been reported thus far.

Therefore, while recent studies see an emerging effort to develop a virtual alternative for cell and tissue culture training, no study thus far has empirically demonstrated the beneficial outcome of VR-assisted training on the learning outcomes of the users. There is also a lack of measurement for other domains of user experience, where most studies only focus on subjective, conscious appraisal towards different aspects of the respective applications.

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3.0 Methodology

With the development of AsepticTech VR application, this research aims to determine whether the application is a feasible teaching and learning tool to achieve the learning objectives for the 'Aseptic technique' topic in the Cell and Tissue Culture course (SBP3410), offered to the Biomedical Science undergraduates in Universiti Putra Malaysia (UPM). This will be addressed by measuring the impact AsepticTech VR application on students' learning performance based on the three learning domains: cognitive, affective, and psychomotor. Additionally, the study also explored the influence of AsepticTech VR on users' emotions. Finally, the study aims to gauge the learning experience associated with the use of AsepticTech VR.

3.1 AsepticTech VR Application

AsepticTech VR was designed on Unity® platform (version 2018.4.13f1) as the intervention in this study. AsepticTech VR is compatible with smartphones using Android operating system 5.1 or above. The application employs gaze-based interaction, in which users interact with the virtual environment by looking and pointing a central reticle at objects for 3 seconds.

As the name suggests, the content in AsepticTech VR puts a heavy emphasis on the aseptic techniques of a cell and tissue culture workflow. The application content is fully developed based on the lecture and practical activities conducted in SBP3410 Cell and Tissue Culture. It comprises six main modules, each of which exposes the users to sets of aseptic techniques dedicated to specific parts in a cell and tissue culture workflow. The six modules included are as follows:

(1) Attire and Personal Protective Equipment (PPE)
(2) Selection of the Appropriate Biosafety Cabinet (BSC)
(3) BSC Preparation for Cell and Tissue Culture Work
(4) Cell and Tissue Culture Contamination
(5) Performing a Subculture
(6) Cleaning Up a BSC

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The main storyline of AsepticTech VR involves users being tasked to perform a subculture of RAW264.7 murine macrophages in a flask. To complete the task, users were guided by detailed instructions as they navigated from one module to another. Besides, during their playthrough, users were assessed via quizzes at specific checkpoints and received feedback to enhance their knowledge on aseptic techniques. Users of AsepticTech VR may also directly enter any one of the six modules from the main menu. As such, the application provides the convenience of directly skipping to a specific module instead of having to explore the whole storyline from the beginning.

A screenshot of the application is shown in Figure 1.

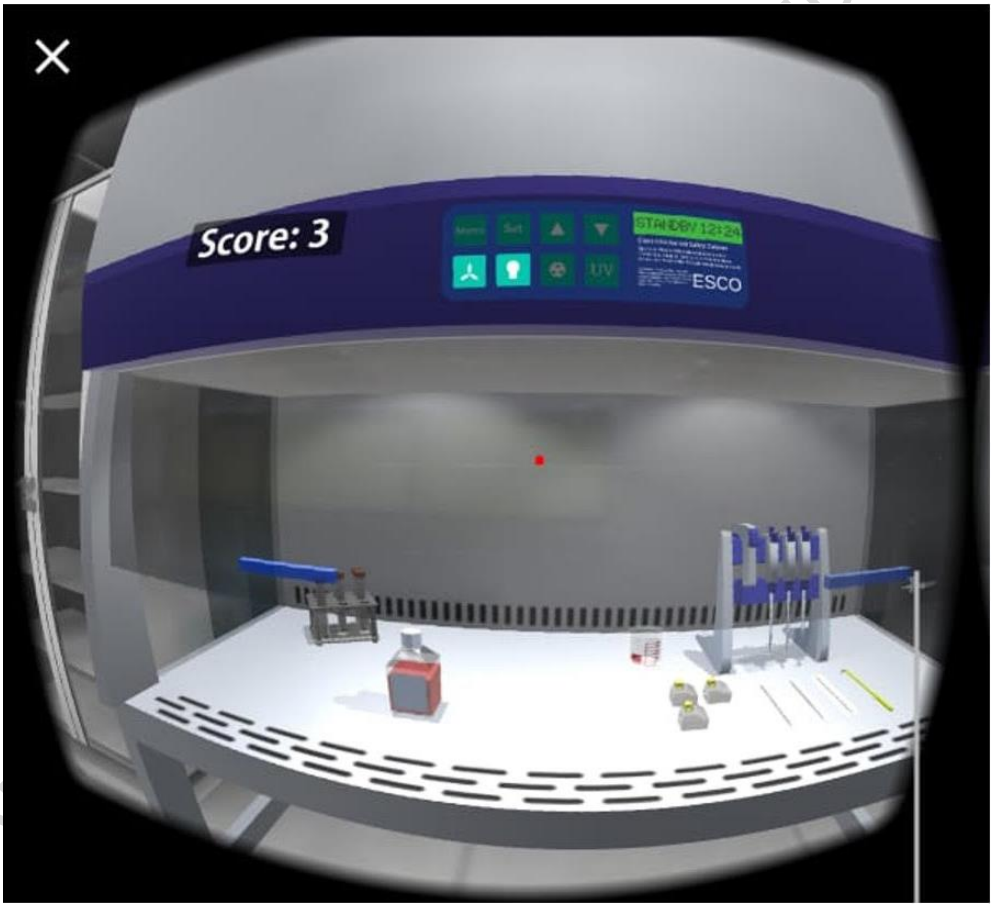

Fig. 1 Screenshot of a Biosafety Cabinet model in AsepticTech VR

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3.2 Hardware

Participants were exposed to virtual reality through the combined use of Virtual Reality Games (VRG) Pro headset and smartphones with Android operating system 5.1 or above.

3.3 Questionnaires and Rubric Development

Prior to the execution of the actual study, questionnaires and rubrics were developed to assess the participants' learning performance, emotions, and experience.

3.3.1 Learning Performances

The first section of the questionnaire aimed to gauge the participants' learning performances, which can be broken down into the three main domains of learning: cognitive, affective, and psychomotor (Enamul, 2016). Therefore, three separate evaluation tools were developed to assess the participants' performance in each learning domain.

In the context of this study, cognitive learning performance refers to the ability to retain, understand, and apply the theoretical knowledge on aseptic techniques from the lecture and practical content of SBP3410 Cell and Tissue Culture course. To assess the participants' learning performances, 10 multiple-choice questions related to aseptic techniques were developed, with each containing 1 correct option and 3 incorrect options. The 10 multiple-choice questions for assessing cognitive learning performance are listed in Online Resource 1.

Secondly, in the context of this study, affective learning performance refers to the affective learning outcomes associated with aseptic techniques, such as appreciation and confidence with regards to the participants' aseptic technique skills. The affective learning performance was assessed using five to eleven Likert scale questions, with each question containing a statement to which participant expresses their degree of agreement (1 = Strongly disagree, 5 = Strongly agree). The items for assessing affective learning performance are listed in Online Resources 2 to 4.

Thirdly, the psychomotor learning performance in this study refers to the participants' ability to follow proper aseptic techniques when performing cell and tissue cultures. In this

study, the participants' psychomotor learning performance was evaluated by carrying out specific cell and tissue culture tasks physically in a laboratory setting based on a given scenario. The participant's ability to adhere to the aseptic techniques was monitored and assessed by an independent evaluator according to a rubric. The rubric for assessing psychomotor learning performance is shown in Online Resource 5.

Collectively, the three subsections described above are referred to as the "learning performance questionnaire and rubric". The learning performance questionnaire and rubric were piloted on five faculty members of FHMS consisting of lecturers involved in the planning and teaching of SBP3410. The questionnaire and rubric were improved based on feedback collected from faculty members before they were applied in this study.

3.3.2 Learning Emotions

The second section of the evaluation tools aimed to quantify the degree of emotions experienced by the users of AsepticTech VR. Specifically, the study intended to measure the strength of positive emotions versus negative emotions associated with the use of AspeticTech VR. To this end, the study adopted the Modified Differential Emotions Scale (mDES) questionnaire developed by Fredrickson (2013). The mDES questionnaire consisted of 20 Likert-scale questions quantifying the strength of 10 different positive emotions and 10 different negative emotions (1 = Strongly disagree, 5 = Strongly agree). One item for positive emotion was omitted due to irrelevance, leaving 9 items for positive emotions and 10 items for negative emotions. The mDES questionnaire employed in this study is listed in Online Resource 6. The mDES questionnaire was only given to the test group participants.

3.3.3 Learning Experience

The third section of the evaluation tool aimed to investigate the learning experience associated with the use of AsepticTech VR. This component was assessed via the adoption of Web-Based Learning Tools (WBLT) questionnaire developed by Kay (2011). The WBLT questionnaire consisted of 13 Likert-scale questions where students rated the VR application's ability to help them in their learning, the application's design, and its engagement level (1 = Strongly disagree, 5 = Strongly agree). In addition, this section also contains two open-ended questions, to which students reflect any positive and negative aspects of the application based on their perspective.

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The WBLT questionnaire employed in this study is listed in Online Resource 7. As with mDES questionnaire, the WBLT questionnaire was also only given to test group participants.

3.4 Participants

A total of 53 active biomedical science undergraduates from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) enrolled in SBP3410 Cell and Tissue Culture were invited to participate in the study voluntarily. The sample population consists of a mix of males and females, with ages ranging from 19 to 23 years. Students also had equal prior exposure to aseptic techniques through traditional lectures and hands-on sessions through SBP3410.

Due to the limited number of students fitting the inclusion criteria as previously described, universal sampling was performed to recruit all 53 undergraduates from the course SBP3410. Students were briefed on the scope and flow of the study. Additionally, to address potential conflict of interest issue, students were also informed that their participation, performance, and feedback in the study would not affect their final grade for the course. A consent form was distributed to students who wished to enroll in the study. Among the 53 students, 41 of them provided their consent to participate in the study. Demographic information on participants' age and sex is displayed in Table 1.

Table 1. Demographic information of study participants.

Characteristics	Data
Mean age (*SD*), in years	20.1 (0.89)
Gender	
Female	29 (70.7%)
Male	12 (29.3%)

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Upon recruitment, participants were randomly divided into 2 groups: the test group or the control group using an online random group generator (Random Team Generator; https://www.randomlists.com/team-generator). Out of 41 participants, 22 were distributed into the test group, and 19 were distributed into the control group. As to be described in the following section, test group participants are participants who were given access to AsepticTech VR as the intervention of interest in this study. Meanwhile, control group participants are those who were not given access to AsepticTech VR during the course of this study.

3.5 Study Design

This study was conducted in the form of a randomized controlled trial divided into three phases: pre-test, intervention, and post-test. The study design is summarized in Figure 2.

3.5.1 Pre-test

In the first phase (pre-test phase), participants from both the control and test groups completed the learning performance questionnaires and a practical assessment, the latter of which was scored based on a rubric by independent enumerators. The scores from the pre-test phase were recorded as the baseline learning performances in the cognitive, affective, and psychomotor learning domains.

3.5.2 Intervention

The intervention phase immediately follows upon completion of the first phase. On the same day, the test group participants who had completed the pre-test were allowed to use the AsepticTech VR application and complete all the designated modules. In contrast, the control group was not given such an opportunity. Following the intervention, both groups were exposed to one week of traditional lectures and practical sessions on aseptic techniques. Additionally, participants from the test group were also able to use the VR application at their convenience upon request within the one-week gap between the pre-test session and post-test session.

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3.5.3 Post-test

After one week, participants from both test and control groups returned for a post-test session. Both groups completed the learning performance questionnaires and were assessed on their practical skills based on a rubric, from which their post-intervention scores were recorded. The post-test was again, conducted by independent enumerators. Moreover, participants from the test group also answered two additional questionnaires: the mDES and WBLT questionnaires, as described prior.

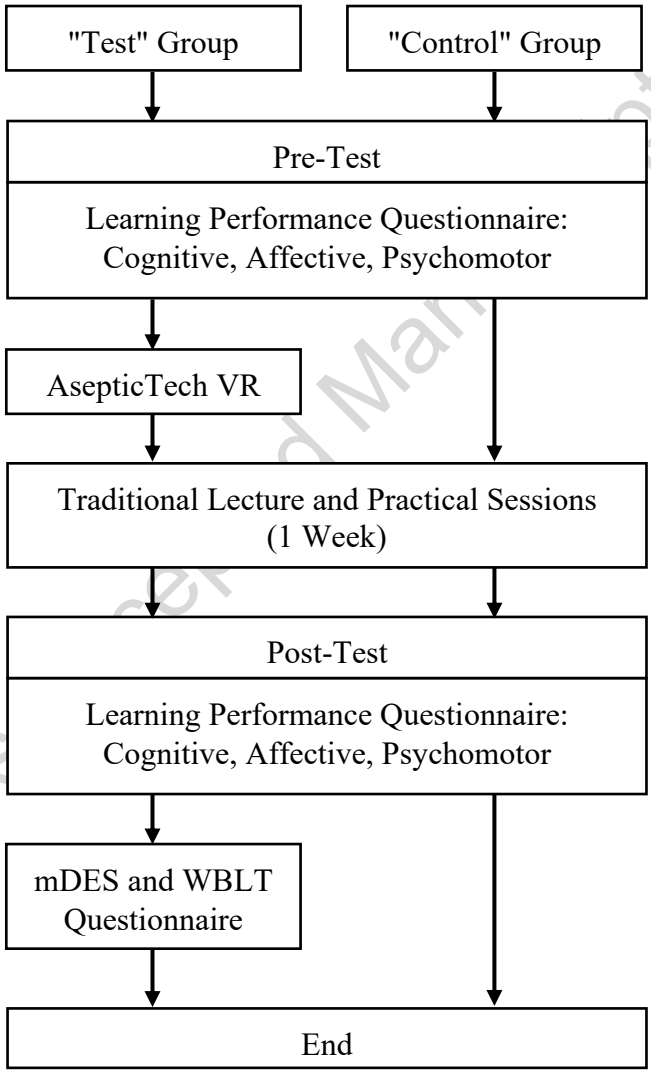

Fig. 2 A flowchart showing the overall flow of the study and the different phases within the study

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3.6 Data Analysis

3.6.1 Learning Performances

To evaluate the differences in learning performances between the two groups, an initial comparison of the baseline cognitive, affective, and psychomotor scores between the test group and the control group was performed using Mann-Whitney U Test and independent t-tests. Subsequently, the improvements in cognitive, affective, and psychomotor scores (represented by the difference in post-test and pre-test scores) were compared between the two groups via the use of Mann-Whitney U Test and independent t-tests as well. The decision between Mann-Whitney U test and independent t-test was determined based on conformation to parametric test assumptions. The data analyses performed using the learning performance questionnaires and rubrics are depicted in Figure 3. Additionally, the affective learning performance scores improvements were also compared between the two groups based on individual questions. Specifically, the score improvement for items LA1 to LA5 (Online Resources 2 to 4) was compared between the test group and the control group to identify specific affective learning outcomes that differ between the two groups.

3.6.2 Learning Emotions

Due to the omission of 1 question for positive emotions as described prior, the score ratings provided by participants in the mDES questionnaire were averaged to allow equal comparison. Specifically, the scores for positive emotions were averaged across 9 items, while the scores for negative emotions were averaged across 10 items. Subsequently, the average scores of positive and negative emotions were compared via a paired t-test to identify whether there is a difference between the positive and negative emotions experienced by AsepticTech VR users.

All statistical analyses were performed using IBM SPSS Statistics (Version 27) with $\alpha = 0.05$.

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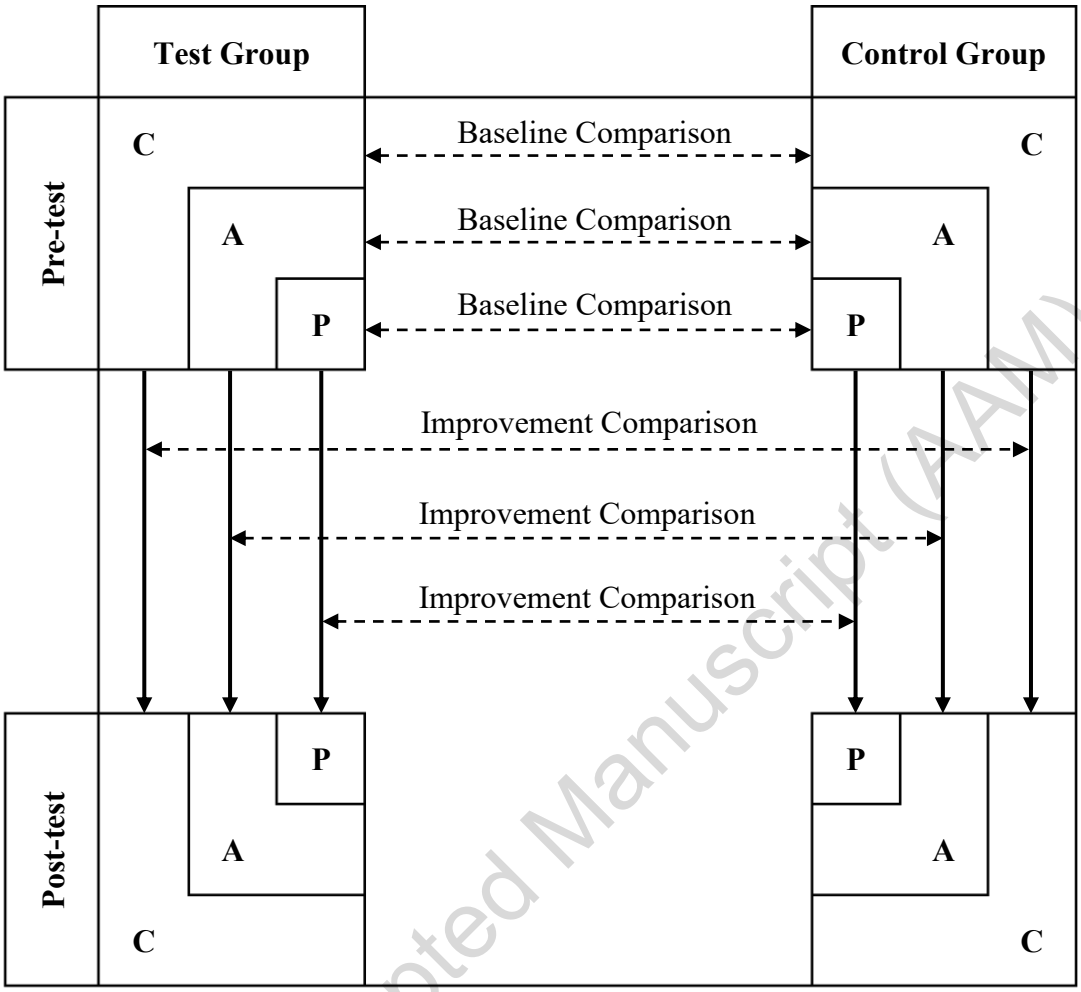

Fig. 3 Summary of data analyses performed on the learning performance questionnaires and rubric (C=Cognitive learning performance, A=Affective learning performance, P=Psychomotor learning performance)

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4.0 Results

The questionnaires and rubrics used in this study yielded results on the learning performance of the test and control groups before and after the one-week gap, alongside the emotions and learning experience of the test group participants related to the use of AsepticTech VR.

4.1 Learning Performances: Cognitive, Affective and Psychomotor

As described in the Methodology, the learning performances of the participants were assessed based on the three learning domains by using the learning performance questionnaires and rubric which consisted of three subsections. Out of 41 participants, all 41 (100%) participants completed the pre-test and post-test questionnaires assessing the cognitive and affective learning performances. Meanwhile, 40 (97.56%) of 41 participants completed the pre-test and post-test simulation tasks assessing their psychomotor learning performances. One participant in the test group failed to complete the pre-test psychomotor assessment due to early termination of the assessment. By using Grubbs' test and constructing a box plot, the psychomotor score of the participant was identified as an outlier for the dataset and was discarded from all subsequent analyses. The descriptive statistics of the learning performance questionnaires and rubric scores are summarized in Table 2, whereas the result of the statistical analysis is summarized in Table 3. The score differences across the three domains are displayed in Figure 4.

Table 2. Descriptive statistics for the learning performance questionnaires and rubric scores. The data is presented as mean ± standard deviation.

Scores	Group	n	Mean (±*SD*)
Pre-Test Affective	Control	19	16.21 (±4.077)
Pre-Test Cognitive	Control	19	4.58 (±1.539)
Pre-Test Psychomotor	Control	19	18.42 (±3.437)
Pre-Test Affective	Test	22	15.14 (±4.190)
Pre-Test Cognitive	Test	22	4.82 (±1.763)
Pre-Test Psychomotor	Test	21	18.29 (±3.019)
Affective Score Improvement	Control	19	2.95 (±3.808)

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Cognitive Score Improvement	Control	19	0.68 (±1.701)
Psychomotor Score Improvement	Control	19	3.58 (±3.934)
Affective Score Improvement	Test	22	5.59 (±4.677)
Cognitive Score Improvement	Test	22	1.95 (±1.430)
Psychomotor Score Improvement	Test	21	7.05 (±4.088)

Table 3. The statistical analyses and result for the learning performance questionnaires and rubric scores.

Score 1	n_1	**Score 2**	n_2	**Statistical Test**	***p***
Test Group Pre-Test Cognitive Scores	22	Control Group Pre-Test Cognitive Scores	19	Mann-Whitney U test	0.9470
Test Group Pre-Test Affective Scores	22	Control Group Pre-Test Affective Scores	19	Mann-Whitney U test	0.4456
Test Group Pre-Test Psychomotor Scores	21	Control Group Pre-Test Psychomotor Scores	19	Independent t-test	0.8952
Test Group Cognitive Scores Improvement	22	Control Group Cognitive Scores Improvement	19	Mann-Whitney U test	0.0081*
Test Group Affective Scores Improvement	22	Control Group Affective Scores Improvement	19	Mann-Whitney U test	0.1033
Test Group Psychomotor Scores Improvement	21	Control Group Psychomotor Scores Improvement	19	Independent t-test	0.0133*

* : Significant difference (p<0.05) between the groups.

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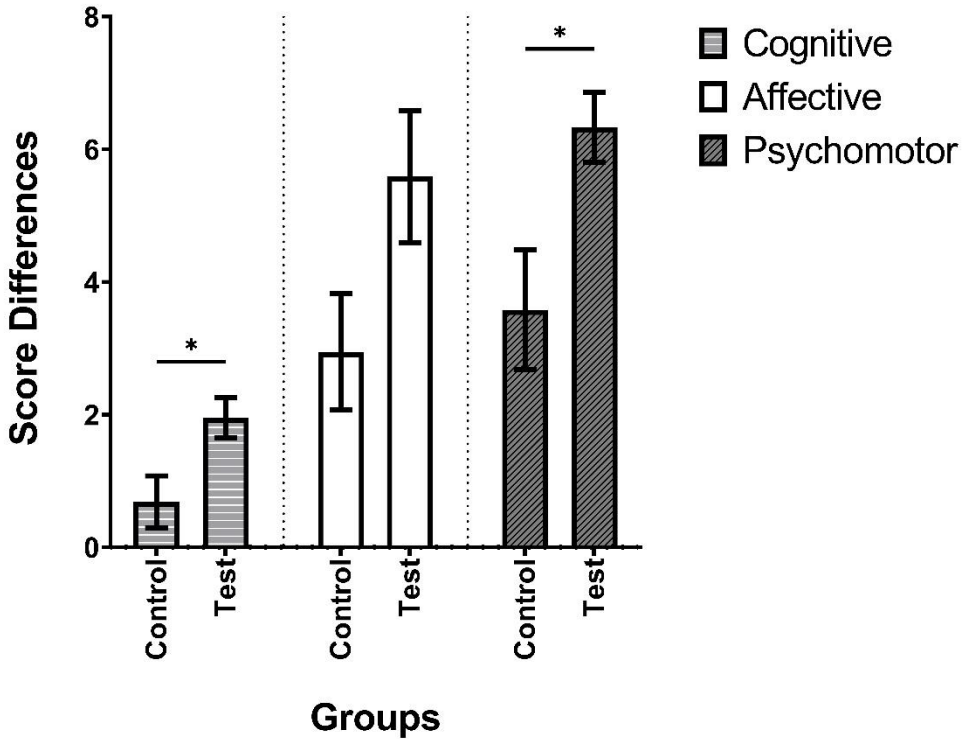

Fig. 4 Learning performance score difference among the control and test groups across the three domains, presented as mean ± SEM; *p<0.05

4.1.1 Comparison of Baseline Learning Performance

The Mann-Whitney U Test did not reveal a significant difference in the median baseline cognitive scores (U=180, p=0.9470) between the test group (median=4.00) and the control group (median=5.00). Besides, the Mann-Whitney U Test also failed to detect a difference in the median baseline affective scores (U=211.5, p=0.4456) between the control group (median=15.00) and the test group (median=14.50). Similarly, the independent t-test did not identify any significant differences between the mean baseline psychomotor scores [t(38)=38, p=0.8952] between the test group (mean=18.33) and the control group (mean=18.67). These results collectively suggest that the two groups did not differ significantly in terms of cognitive, affective, and psychomotor learning performance prior to the intervention. Therefore, the two groups are assumed to be equal and suitable for future comparisons.

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4.1.2 Comparison of Learning Performance Improvement

For the comparison of improvement scores, Mann-Whitney U test revealed a significant difference between the median cognitive score improvement (U=308.5, p=0.0081) of the test group (median=2.00) and the control group (median=0.00). This suggests that the test group participants had better improvements in cognitive learning performance compared to the control group. Similarly, the independent t-test suggests a significant difference in the mean psychomotor score improvement [t(29.301)=38, p=0.0133] following the intervention between the test group (mean=6.33) and control groups (mean=3.58). This also suggests that the control group participants had better improvements in their psychomotor learning performance compared to the control group. However, the Mann-Whitney U Test did not provide enough evidence to suggest a difference in median affective score improvement (U=271, p=0.1033) between the test (median=5.00) group and the control group (median=5.00). Therefore, the results suggest that both the test and control groups had similar affective score improvements.

A deeper analysis of the five items assessing affective learning performance, specifically items LA1 to LA5 (Online Resources 2 to 4) was performed to identify differences in improvements in any specific affective learning outcomes between the test and control group. Mann-Whitney U Test was performed to detect significant differences between improvement in scores across each item from LA1 to LA5. The analysis revealed a significant difference in only 1 out of the 5 items. Specifically, the test group scored a higher median score (median=1.50) as compared to the control group (median=1.00) for item LA1 [U=298.5, p=0.015]. Other than that, there were no significant differences between the two groups for the remaining four items (Table 4).

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Table 4. A further analysis on items LA1 to LA5. Only responses for LA1 showed a significant improvement in the test group as compared to the control group.

		Median Score Improvement				
Code	**Question**	**Test**	n_1	**Control**	n_2	p
LA1	I feel confident in my level of knowledge regarding aseptic techniques that are needed during cell culture work.	1.50	22	1.00	19	0.0153*
LA2	I can use aseptic techniques when performing cell culture work in a biosafety cabinet.	1.00	22	1.00	19	0.1443
LA3	I feel comfortable performing cell culture in a biosafety cabinet using aseptic technique.	1.00	22	0.00	19	0.1907
LA4	I can ensure the quality of my workflow when dealing with a biosafety cabinet in a cell and tissue culture laboratory.	1.00	22	1.00	19	0.2279
LA5	I am confident that I would be able to conduct cell culture by using proper aseptic technique.	1.00	22	1.00	19	0.4162

* : Significant difference (p<0.05) between the groups.

During the post-test, the test group participants were given six additional questions specifically on AsepticTech VR as part of the post-test affective learning performance questionnaire. The descriptive statistics of the responses are summarized in Table A1. Interestingly, the participants from the test group reflected a high affective learning performance score for LA6-11 (at least 4.409 out of 5.00) during the post-test despite having no significant difference between the control and test groups on items (LA1 to LA5) as described earlier. This indicates the test group participants found AsepticTech VR useful in increasing their confidence in terms of knowledge and practical skills.

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4.2 Learning Emotions: Positive and Negative Emotions

All 22 test group participants (100.00%) also completed the designated mDES questionnaire during their post-test. The descriptive statistics of the mDES questionnaire are summarized in Table 5. The paired t-test showed a significant difference between the mean positive emotions and negative emotions (mean difference=1.869) experienced by the test group participants associated with the use of the AsepticTech VR application [$t(21)=10.546$, $p<0.001$]. Based on the descriptive statistics in Table 5, the three strongest positive emotions (in descending order based on mean ±SD scores) are "grateful, appreciative, or thankful", "joyful, glad, or happy", and "hopeful, optimistic, or encouraged". In contrast, the three strongest negative emotions (in descending order) are "stressed, nervous, or overwhelmed", "scared, fearful, or afraid", and "guilty, repentant, or blameworthy".

Table 5. Results from the mDES questionnaire. The table shows the type of emotion indicated by the respective questions, and the mean ± standard deviation of responses for each item.

Code	Question	Emotion Type	Mean (±*SD*)
DE1	What is the most amused, fun-loving or silly you felt?	Positive	3.000 (±0.690)
DE2	What is the most angry, irritated or annoyed you felt?	Negative	1.273 (±1.162)
DE3	What is the most ashamed, humiliated or disgraced you felt?	Negative	1.182 (±1.220)
DE4	What is the most awe, wonder or amazement you felt?	Positive	3.182 (±0.733)
DE5	What is the most contemptuous, scornful or disdainful you felt?	Negative	1.000 (±0.873)
DE6	What is the most disgust, distaste or revulsion you felt?	Negative	0.727 (±0.935)
DE7	What is the most embarrassed, self-conscious or blushing you felt?	Negative	1.545 (±1.405)
DE8	What is the most grateful, appreciative or thankful you felt?	Positive	3.636 (±0.727)
DE9	What is the most guilty, repentant or blameworthy you felt?	Negative	1.636 (±1.399)

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DE10	What is the most hate, distrust or suspicion you felt?	Negative	0.727 (±1.032)
DE11	What is the most hopeful, optimistic or encouraged you felt?	Positive	3.409 (±0.796)
DE12	What is the most inspired, uplifted or elevated you felt?	Positive	3.227 (±1.020)
DE13	What is the most interested, alert, curious you felt?	Positive	3.364 (±0.954)
DE14	What is the most joyful, glad or happy you felt?	Positive	3.500 (±0.673)
DE15	What is the most proud, confident or self-assured you felt?	Positive	2.909 (±0.921)
DE16	What is the most sad, downhearted or unhappy you felt?	Negative	1.318 (±1.171)
DE17	What is the most scared, fearful or afraid you felt?	Negative	1.682 (±0.946)
DE18	What is the most serene, content or peaceful you felt?	Positive	2.500 (±1.102)
DE19	What is the most stressed, nervous or overwhelmed you felt?	Negative	2.136 (±1.125)
	Average of Positive Emotions		**3.192 (±0.526)**
	Average of Negative Emotions		**1.323 (±0.682)**

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4.3 Learning Experience: Learning, Design and Engagement of AsepticTech VR

All 22 test group participants (100.00%) completed the designated WBLT questionnaire during their post-test. The descriptive statistics of the WBLT questionnaire are summarized in Table 6. Collectively, the participants rated the AsepticTech VR highly in terms of learning experience, where all three fields had a mean score higher than 4. Specifically, the learning aspect of the application was rated the highest (mean=4.709), followed by its engagement construct (mean=4.577), and tailed by the design construct (mean=4.227).

Table 6. Descriptive statistics of the WBLT questionnaire responses. The data is presented as mean ± standard deviation.

Code	Question	Mean (±*SD*)
Learning		**4.709 ±0.389**
WL1	Working with AsepticTech VR helped me learn.	4.864 ±0.351
WL2	The feedback from AsepticTech VR helped me learn.	4.636 ±0.492
WL3	The graphics and animations from AsepticTech VR helped me learn.	4.591 ±0.503
WL4	AsepticTech VR helped teach me a new concept.	4.682 ±0.567
WL5	Overall, AsepticTech VR helped me learn.	4.773 ±0.428
Design		**4.227 ±0.634**
WD1	The help features in AsepticTech VR were useful.	4.318 ±0.779
WD2	The instructions in AsepticTech VR were easy to follow.	4.364 ±0.726
WD3	AsepticTech VR was easy to use.	4.000 ±0.816
WD4	AsepticTech VR was well organized.	4.136 ±0.833
Engagement		**4.577 ±0.596**
WE1	I liked the overall theme of AsepticTech VR.	4.591 ±0.590
WE2	I found AsepticTech VR engaging.	4.591 ±0.796
WE3	AsepticTech VR made learning fun.	4.636 ±0.658
WE4	I would like to use AsepticTech VR again.	4.455 ±0.738

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4.3.1 Qualitative Feedbacks

The WBLT questionnaire also gathered positive and negative points regarding the AsepticTech VR applications from the test group participants. Table 7 below shows a summary of the main points submitted as well as examples of indicative quotes.

Table 7. Qualitative feedback regarding the positive and negative points of AsepticTech VR.

Item	Main Points and Indicative Quotes
Please report the positive points of the experience you had using AsepticTech VR.	(1) AsepticTech VR introduces users to new concepts. "*AsepticTech VR introduces me to so many concepts available when working with cell cultures, and it prepares me to work properly on the actual cell culture tasks.*" (2) AsepticTech VR helped users to better remember the workflow of cell culture. "*AsepticTech VR helped me to recall all the knowledge that I learned during online classes. It gave me a real picture of working in a cell culture laboratory. Before this, I just watched YouTube videos to learn a proper aseptic technique, but I tend to forget what I learn.*" (3) AsepticTech VR provides participants a better understanding towards cell culture workflow. "*It is also a good choice to use this app before the actual physical tissue culture experiment to strengthen the memory on the knowledge and steps of experiments.*" (4) AsepticTech VR provides users an opportunity to experience performing cell culture.

	"*This app allows me to experience doing cell culture work in BSC even though it is not physically it feels like I'm doing it physically.*"
	(5) AsepticTech VR made learning process fun and engaging. "*Easy to use and it made the learning process becomes fun.*"
	(6) AsepticTech VR has a good design. "*The graphic is really beautiful*." "*The content and graphic are very nice and helpful*."
	(7) AsepticTech VR provides easy-to-follow instructions. "*The graphics are fun, and the instructions are easy to follow along and for somebody who never experienced working in a biosafety cabinet this VR definitely created a wonderful experience.*"
Please report the negative points of the experience you had using AsepticTech VR.	(1) Participants reported experiencing nausea and discomfort while using AsepticTech VR. "*I experienced motion sickness during the VR session. I felt dizzy, uncomfortable, and stressed out after module 3.*"
	(2) Participants faced difficulties in aiming the reticle to interact with objects in the application. "*The reticle was not accurate sometimes, so it was hard for me to point at some items, especially fine objects (serological pipette, scrapper etc.).*"
	(3) AsepticTech VR requires long waiting time (3 seconds) for interaction to occur. "*In addition, the reticle was unable to sense the answer sometimes. I had to try many times to adjust the reticle to the answer, but it took a long time to sense it.*"

(4) The resolution of AsepticTech VR is low.

"*The resolution of the VR app is a bit low thus making the images look like pixels when moving from one place to another. Pixelized images made my head hurt*."

(5) Participants with vision problems face difficulties in achieving optimum clarity.

"*I think the VR is not so suitable for those who are having myopia. Without the spectacles, I was still able to see the words, but they were a bit blurry to me*."

"*Since I have astigmatism, it's quite difficult for me to see the VR screen properly. All the pictures became double, so I found it was quite difficult to aim the reticle for 3 seconds*."

(6) The storyline design in AsepticTech VR limits player autonomy.

"*No autonomy/freedom over what I want to do, and the course is limited to the module as of the last experience I had with the app*."

(7) Participants experienced blurring lenses while using the application with a mask on.

"*The lenses turned hazy during exhalation while wearing the headset*."

"*The VR lenses became blurry after using a long time due to evaporation*."

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5.0 Discussion

This research aimed to investigate whether AsepticTech VR can be proposed as a useful tool to assist teaching and learning of aseptic techniques in cell and tissue culture among biomedical science undergraduates. Therefore, three aspects of the application were studied, namely its impact on learning performance, its influence on learning emotions, and the learning experience provided.

Forty-one biomedical science undergraduates currently enrolled in SBP3410 Cell and Tissue Culture course were recruited and randomized into a test group and a control group. In this study, participants in the test group were exposed to AsepticTech VR in addition to traditional teaching methods, which included lectures and practical sessions. Meanwhile, the control group participants were only exposed to traditional teaching and learning activities (Figure 2). AsepticTech VR was hypothesized to positively impact cognitive, affective, and psychomotor learning performances among the test group participants compared to the control group. Analysis of the score improvements in the learning performance questionnaires and rubric revealed that the test group participants had significantly better improvements in cognitive and psychomotor scores following the use of AsepticTech VR as compared to the control group. Therefore, this finding partially supports the experimental hypothesis. This result is concurrent with previous reports which have been summarised in a comprehensive review by Jensen and Konradsen (2017), where the use of VR can generally improve learning outcomes.

However, our study did not reveal a significant effect of the AsepticTech VR application on the affective domain learning performances. This suggests that AsepticTech VR application may not be effective in enhancing affective domain learning performances. Analysis by individual questions only revealed a significant difference in one of the five affective learning performance questions between the test and control groups. In other words, though the application increased the confidence of users towards their cell and tissue culture knowledge (LA1), the level of confidence in their practical skills did not differ from those of the non-users (LA2 to LA5). This finding contradicted the previously established mechanism which reported that VR was able to improve affective learning outcomes by increasing the learners' presence, which is an antecedent to learning enjoyment and motivation (Makransky & Lilleholt, 2018). Nevertheless, a paper by Shorey and Ng (2021) reported a similar finding,

where immersive VR system did not result in improved affective learning outcome in nursing education. One possible explanation for this is the lack of control and active learning in the VR app itself (Makransky & Lilleholt, 2018). Indeed, the design of the VR application was such that users would follow a set of instructions throughout the session, guided from one module to another. The presence of step-by-step guidance may have negatively impacted control and active learning, hence limiting affective learning improvement (Pekrun & Stephens, 2010). Besides, another possible explanation may be the lack of realism (Shorey & Ng, 2021), since AsepticTech VR application employed a gazed-based interaction method, where interaction with the virtual environment was done by pointing a reticule at an object via head movements. Such design possibly decreased the realistic experience as participants may navigate through the virtual simulation without requiring hand movements. Together, the lack of control and lack of realism possibly led to a lower degree of presence in the virtual simulation (Makransky & Lilleholt, 2018). Furthermore, as quoted by Lu et al. (2018), the common adverse health effects related to the use of VR could also be a contributing factor that decreases the sense of presence among VR users. This is in line with the present findings, as indicated by a few comments in the qualitative feedback reflecting nauseous feelings and discomfort while using AsepticTech VR. Nonetheless, the positive responses for items LA6 to LA11 (Table A1) may indicate that the use of AsepticVR had an overall positive impact on affective learning performances.

This study further investigated the emotional experience of AsepticTech VR users via the use of mDES questionnaire, due to the established positive relationship between emotions and learning. The analysis revealed that AsepticTech VR users have significantly stronger positive emotions as compared to negative emotions, which is concurrent with previous studies (Allcoat & von Mühlenen, 2018, Um et al., 2012). Based on their responses, AsepticTech VR users were not only thankful and happy for the opportunity to use the VR application as a medium to learn, but more importantly encouraged to learn using AsepticTech VR. This suggests that AsepticTech VR is appropriately designed to enhance positive emotions among the users and may possibly increase motivation and satisfaction among users to increase their desire to learn. The final part of the study investigated the users' learning experience associated with AsepticTech VR. The learning experiences were scrutinised in terms of thee constructs, namely the application's usefulness to help participants learn (Learning), the application's design (Design), and the degree to which the application is engaging (Engagement). Results from the WBLT questionnaire revealed very high ratings on the application's learning, design,

and engagement constructs, with a satisfactory mean score of higher than 4 for all three constructs. A similar rating was reported by Kaltsidis et al. (2021) when WBLT questionnaire was used to evaluate the learning experience of users on a similar application named LabTraining VR: Biosafety Cabinet Edition. This may imply that both applications provide comparable positive learning experience to the users and are equally suitable to be used as a teaching and learning tool in a classroom setting.

LabTraining VR: Biosafety Cabinet Edition is an aseptic technique VR application developed by the Centers for Disease Control and Prevention (CDC) that was launched in 2020. On the other hand, AsepticTech VR was developed in-house since 2019 and completed in 2021. Even though AsepticTech VR may bear much contextual resemblance to LabTraining VR: Biosafety Cabinet Edition, AsepticTech VR may be more advantageous in terms of cost, convenience, accessibility, and content. Cost-wise, LabTraining VR by CDC is only compatible with HTC Vive (priced around USD1600) or Oculus Quest 2 VR equipment (priced around USD280), coupled with a laptop. By comparison, AsepticTech VR only requires an Android mobile phone and a headset for use (pricing of the headset can go as low as USD6). Since AsepticTech VR has a simpler hardware requirement, it would be more accessible to students from diverse socioeconomic backgrounds. Furthermore, this accessibility could potentially encourage students to use the application at home, as well. By allowing students to use the application outside of the classroom, AsepticTech VR may provide a valuable resource for enhancing learning outcomes.

Content-wise, compared to LabTraining VR: Biosafety Cabinet Edition, which focuses solely on the handling of BSCs, AsepticTech VR offers a broader range of learning modules. In addition to BSC training, AsepticTech VR covers the entire workflow of working in a cell and tissue culture laboratory, from entering to exiting. These modules were developed based on SBP3410 and are directly applicable to biomedical undergraduates in FHMS, allowing them to apply their knowledge and improve their learning outcomes.

The positive comments garnered via the open-ended qualitative feedback questions correlated well with the ratings for the three constructs in WBLT, where the positive feedback mainly commended on AsepticTech VR's ability to help them learn, its engaging nature, alongside some of its design aspect. On the contrary, the negative comments reported experience of physical discomfort such as nauseous feeling and dizziness which is common

among previous studies involving VR (Birbara et al., 2019; Saredakis et al., 2020 ; Fussell & Truong, 2021; Kaltsidis et al., 2021). The top negative emotion experienced, namely "stressed, nervous and overwhelmed" may have been a result of the adverse health effect experienced by the participants. However, despite these effects, the use of AsepticTech VR still effectively improved cognitive and psychomotor learning performances, while creating a net positive experience for users. Negative feedback about AsepticTech VR's design also surfaced, indicating areas for improvement in future development efforts.

Nevertheless, this study has several limitations that need to be addressed in future research. Firstly, the limited sample size of the study population may be insufficient to generalise the findings to a larger population. Future studies should aim at extending the research to a larger population. Secondly, the study relied on self-reported questionnaire to gather input on participants' emotions. Such method may result in inconsistencies as the emotions may not accurately reflect those that are experienced during the use of AsepticTech VR. Alternative methods that could provide more accurate data on emotions experienced during VR use can be applied in future research. For instance, physiological signals such as brainwaves can be measured (Riva et al., 2007).

Thirdly, this study adopts a relatively short follow-up period, which is a common experimental design adopted by similar previous studies. A number of studies that investigated the effectiveness of VR-assisted learning had relatively short follow-up periods timeframe and concluded that VR-assisted learning is effective in improving conceptual or procedural knowledge (Ekstrand et al., 2018; Maresky et al., 2019; Tergas et al., 2013). However, it is important to note that the participants in this study had prior exposure to aseptic technique knowledge and skills through traditional lectures and practical sessions. This study does not conclude that AsepticTech VR can be used to train aseptic techniques on someone with no background at all. Indeed, aseptic techniques in cell and tissue culture are more than just performing a series of procedures to prevent contamination, but also involve intricate planning-ahead and dexterity, such that it becomes a second nature while dealing with a sterile environment. Therefore, another study with a longer follow-up period may be conducted in the future to examine the long-term effect of AsepticVR on users' learning performance.

Despite AsepticTech VR in this current form does not offer hands-on movement, this VR application can still enhance cell and tissue culture aseptic technique training through

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immersive and interactive simulations. It provides highly detailed 3D visualizations of cell culture equipment, environments, and procedures. Users can explore virtual laboratories, interact with equipment, observe cell culture processes, and perform them in real-time without physically moving their hands. Also, VR platforms can offer interactive learning modules where users can engage with virtual equipment, perform tasks, and manipulate virtual objects using controllers or other input devices. This allows users to practice cell culture techniques, such as pipetting, media preparation, and cell seeding, in a simulated environment. Nevertheless, as this is the first study that applies VR training in the context of cell-and-tissue culture, the findings in this study could therefore be regarded as preliminary results that could warrant future studies with longer duration to truly assess the retention of these improvements. Hence, we propose that AsepticTech VR serves as an effective virtual reality (VR) application that enhances the traditional teaching of aseptic techniques. It seamlessly transitions students from learning cell culture techniques via lectures and practicing cell culture techniques on an open bench to a real cell culture facility using a BSC.

6.0 Conclusion

In conclusion, this study demonstrated that AsepticTech VR is effective in enhancing students' learning performances on cell and tissue culture aseptic technique. AsepticTech VR also elicited positive emotions among users and provided a superior learning experience, which may be associated with the increased learning performance observed prior.

Taken together, these findings suggest that AsepticTech VR is a feasible addition to teaching and learning activities on aseptic techniques and a valuable low-cost training tool for trainees working in a cell and tissue culture settings. This therefore has the potential to save costs in terms of infrastructure and consumables. More importantly, the development of this application may potentially contribute to more rigor research process, as a lower risk of contamination among all future practitioners may translate into better research outcomes with low risk of confounds due to contamination. The findings from this study could also warrant the addition of VR into the cell-and-tissue-culture modules among undergraduate bioscience curriculum in order to bring about better learning outcomes.

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Availability of Data and Materials

The data collected is available from the corresponding author upon request.

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Appendix

Table A1. Descriptive statistics for the six additional affective learning performances questions exclusively given to the test group participants. The data is presented as mean ± standard deviation.

Code	Question	n	Mean (±*SD*)
LA6	AsepticTech VR application improved my knowledge regarding aseptic techniques that are needed during cell culture work.	22	4.591 (±0.5032)
LA7	AsepticTech VR application will help me to use aseptic techniques when performing cell culture work in a biosafety cabinet.	22	4.500 (±0.5118)
LA8	AsepticTech VR application makes me feel more comfortable in performing cell culture in a biosafety cabinet using aseptic technique.	22	4.409 (±0.6661)
LA9	AsepticTech VR application will help me improve the quality of my workflow when dealing with a biosafety cabinet in a cell and tissue culture laboratory.	22	4.636 (±0.4924)
LA10	I feel more confident that I would be able to conduct cell culture by using proper aseptic technique after using the AsepticTech VR application.	22	4.591 (±0.5032)
LA11	I would use the AsepticTech VR application on my own time if I had access to it.	22	4.455 (±0.7385)