

Study on Adenovirus Infection in vitro with Nanoself-Assembling Peptide as Scaffolds for 3D Culture

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Purpose: To construct a three-dimensional (3D) culture model of adenovirus in vitro using the nanoself-assembling peptide RADA16-I as a 3D cell culture scaffold combined with virology experimental technology to provide a novel research method for virus isolation and culture, pathogenesis research, antiviral drug screening and vaccine preparation.

Methods: The nanoself-assembling peptide RADA16-I was used as a 3D scaffold material for 293T cell culture, and adenovirus was cultured in the cells. The growth, morphological characteristics and pathological effects of 3D-cultured 293T cells after adenovirus infection were observed with an inverted microscope and MTS. The proliferation of adenovirus in 293T cells was observed by TEM and detected by qPCR. The levels of TNF- α and IL-8 secreted by adenovirus-infected 293T cells in the RADA16-I 3D culture system were detected by ELISA.

Results: The 293T cells grew well in the RADA16-I 3D culture system for a prolonged period of time. The adenovirus infection persisted for a long time with multiple proliferation peaks, which closely resembled those of in vivo infections. The adenovirus virions amplified in the 3D system remained infectious. There were multiple secretion peaks of TNF- α and IL-8 secretion levels in adenovirus-infected 293T cells cultured in 3D culture systems.

Conclusion: The nanoself-assembling peptide RADA16-I can be used as a 3D scaffold for adenovirus isolation, culture and research. The 3D culture system shows more realistic in vivo effects than two-dimensional (2D) culture.

Keywords: nanoself-assembling peptide, 3D culture, adenovirus, 293T cells

Introduction

Infectious diseases pose a great threat to public health. Among the pathogens that cause infectious diseases, viruses from either zoonotic sources or vector-borne sources are the main pathogens that result in the most pandemic threats.¹ There are few effective drugs to treat viral diseases, which are highly contagious and widespread. In addition to causing an acute infection, some viruses can cause a persistent infection, and certain viruses are even closely associated with the occurrence of tumours and autoimmune diseases.^{2,3} Therefore, research on the physical and chemical properties, pathogenic mechanisms, immune mechanisms and antiviral drugs for the treatment of viruses has become one of the hotspots in medical and life science research. Viruses can grow only in living cells because of the lack of genetic information-encoding elements required for energy metabolism or protein synthesis (mitochondria and ribosomes), so it is more difficult to culture

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viruses than other microorganisms that can grow on inanimate media. Currently, virus culture methods include animal inoculation, chick embryo cultivation and cell culture, wherein cell culture is the most commonly used method for virus isolation, identification and research.⁴

Viruses are mostly cultured *in vitro* in two dimensions in glass or plastic dishes. Many cells gradually planarize, differentiate and lose their differentiated phenotypes after being separated from tissues and cultured in two dimensions; in fact, the two-dimensional (2D)-culture method cannot simulate the interaction between cells and the extracellular matrix (ECM) with a spatial structure.⁵ Over decades, researchers have gradually realized that various signals, such as adhesion between cells and the ECM, cytokines, and neurotransmitters in the extracellular microenvironment, actively participate in regulating cell growth, differentiation, proliferation and apoptosis.⁶ Therefore, cell culture technology has continuously improved with the progression from 2D to three-dimensional (3D) and static 3D to dynamic 3D cultures. Three-dimensional cell culture technology has been widely used in tissue engineering, regenerative medicine and *in vitro* studies of tumour cells.^{7–13} In recent years, some scholars have successfully developed 3D cell models for virus culture,^{14–20} and the observed infection efficiency of 3D cell culture is higher than that of 2D single-layer cell culture models.^{18,19} However, there have been few reports on the application of 3D cell culture techniques for virus research.

Currently, knowledge of viral replication, pathogenicity, and drug screening is mostly obtained through 2D cell cultures and animal experiments, whereas cells that grow in tissues *in vivo* live beyond all doubt in a 3D microenvironment, with which the occurrence and development of viral infections are closely associated in the body. Traditional 2D cell culture methods cannot reflect the influence of the microenvironment on the occurrence and development of viral infection *in vivo*. Thus, a 3D culture model that simulates the environment *in vitro* will facilitate the in-depth exploration of viral infection, replication, and pathogenesis and the relationship between the virus and the host. In our previous research work, some 3D cell culture models were successfully established using a nanoself-assembling peptide.^{20,21} Herein, a new project based on the 3D cell culture model is proposed. The aim of this project is to construct a 3D virus culture model using a nanoself-assembling peptide RADA16-I by combining virology technology and nanoself-assembling peptide cell

culture technology to provide a novel theoretical and experimental basis for the study of viral diseases and the development of antiviral drugs and vaccines.

Materials and Methods

Materials

The 293T cell line was purchased from CCTCC (Wuhan Province, China). Adenovirus (Adenovirus-EGFP) was purchased from Shanghai Genechem Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum were purchased from GIBCO (USA). MTS was purchased from Promega (USA). Calcein-AM (Ca-AM), propidium iodide (PI), and DAPI were purchased from Sigma (USA). The nanoself-assembling peptide RADA16-I (Ac-RADARADARADARADA-CONH₂, purity 99%) was synthesized by Shanghai Biotech Bioscience & Technology Co., Ltd. (Shanghai, China). Enzyme-linked immunosorbent assay (ELISA) kits for tumour necrosis factor (TNF)- α and interleukin (IL)-8 were purchased from the Cloud Clone Corp (Wuhan Province, China).

Material Characterization

Scanning Electron Microscopy (SEM) Measurements

For SEM, the RADA16-I hydrogel was prepared to 0.5% weight/volume (wt/vol) and washed. The 0.5% RADA16-I hydrogel was fixed with 2.5% (v/v) glutaraldehyde for 2 hours at 37°C and dehydrated in 70%, 80%, 90%, and 100% ethanol. The critical point drying was used to dry the samples. Finally, the samples were mounted on SEM stubs using a double-coated carbon-conductive tape for SEM (Hitachi, S3400N, Japan) observation.

Transmission Electron Microscopy (TEM) Measurements

For TEM, the RADA16-I peptide solution was diluted to 0.05% weight/volume (wt/vol). Before observation, 0.05% RADA16-I peptide solution was dropped on the surface of a copper grid and negatively stained with 3% phosphotungstic acid for 3–5 minutes. After being dried under an incandescent lamp for a few minutes, the grid was observed directly by TEM (JEOL-JEM123, Japan).

3D Culture of 293T Cells

293T cells seeded in 25-cm² culture flasks were grown at 37°C in a humidified atmosphere containing 5% CO₂ in DMEM supplemented with 10% foetal calf serum. When the density reached 90%, the cells were detached using Trypsin-EDTA 0.05%. Subsequently, they were resuspended in 10%

sucrose, and 75 μL of a cell suspension containing 5×10^4 cells was added to 75 μL of 1% RADA16-I, gently mixed, deposited in a 24-well plate with complete medium and incubated at 37°C with 5% CO_2 for 30 minutes before the first medium change. Twenty-four hours later, the medium was changed again. Afterwards, the medium was changed every 2 d. For some experiments, 3D cultures were scaled to a 96-well plate using 25 μL of cell suspension containing 1.67×10^4 cells and 25 μL of 1% RADA16-I.

MTS Proliferation Assay of 3D-Cultured 293T Cells

3D-cultivated cells were incubated in 96-well plates and 20 μL of MTS reagent was added directly to the wells at 1, 3, 5, ..., and 23 days after cultivation. Then, cell samples were incubated at 37°C for 2 hours in the dark and the absorbance was measured at 490 nm.

Ca-AM, DAPI and PI Staining of 3D-Cultured 293T Cells

Cells grown in 3D conditions in 24-well plates were observed by fluorescence microscopy after staining with Ca-AM, DAPI and PI at 0, 5, 10, 15, and 30 days after cultivation.

Adenovirus Infection of 3D-Precultured 293T Cells

For infection experiments, cells grown in RADA16-I were incubated for 24 hours in a 24-well plate, as previously described. Then, the cells were inoculated with adenovirus (4×10^6 copies viral nucleotides per well) for 24 hours at 37°C . Afterwards, the solution was removed, and cell-laden 3D cultures were washed three times with PBS and covered with complete medium. Observations were carried out every day after infection.

MTS Proliferation Assay of Adenovirus-Infected 3D-Cultured 293T Cells

Cells seeded in RADA16-I in a 96-well plate were infected with adenovirus (1×10^6 copies viral nucleotides per well). Afterwards, 20 μL of MTS reagent was added to the wells at 1, 3, 5, ..., and 23 dpi and the absorbance was detected as previously mentioned.

Enhanced Green Fluorescent Protein (EGFP) Assay for Adenovirus Proliferation in the 3D Culture System

3D-cultured 293T cells in 24-well plates were infected with adenovirus as previously described. The EGFP reporter gene was used as a label to detect virus proliferation in 293T cells under direct fluorescence microscopy at

every second day at 2–22 dpi (3 fields were counted per well with 6 duplicates).

TEM for Adenovirus in 3D and 2D Conditions

Cells cultured in 3D conditions in a 24-well plate were infected with adenovirus as previously described, and the cell cultures (293T cells and hydrogel) were harvested at 9 and 15 dpi. Afterwards, the samples were post-fixed using 1% osmic acid at 4°C for 3 hours, dehydrated through a graded series of alcohol, embedded with Spurr resin, and cut into ultra-thin sections (thickness 70 nm). Specimens were then stained with uranyl acetate and lead citrate, and images were obtained using a JEM1230 TEM (JEOL, Japan) at an acceleration voltage of 80 kV. For 2D samples, the 293T cells were harvested at 3 dpi and observed as previously described.

DNA Extraction and Quantitative qPCR of the 3D-Cultured Adenovirus

3D-cultured 293T cells in 96-well plates were infected with adenovirus (1×10^6 copies viral nucleotides per well), and the cell cultures (supernatant and hydrogel) were harvested at 1, 3, 5, and 23 dpi and stored at -20°C . Subsequently, viral nucleic acids were extracted with a DNA/RNA extraction kit (Tiangen DP315) according to the manufacturer's protocol. qPCR systems were prepared using a BIO-RAD qPCR kit in accordance with the manufacturer's protocol. The primers used for detection were as follows:

EGFP, 5'-TTCAAGATCCGCCACAACA-3' and 5'-CGCTTCTCGTTGGGGTC-3'. The qPCR conditions were as follows: pre-denaturation at 95°C for 2 minutes, 39 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 30 s and a melting curve temperature between 65°C and 95°C . Negative (water) and positive (adenovirus standard samples) controls were established for each amplification. After the reaction was complete, the system automatically generated a standard curve and calculated the copy number of the complete viral genome in the corresponding samples.

Infectivity Detection of Adenovirus Produced in the 3D Cell Cultures

The cell supernatants of 3D cell cultures were collected at 6 dpi (1×10^7 copies based on the qPCR amplification quantification) and then used to infect 293T cells grown in 2D culture. The supernatants and cells of 2D culture

were collected at 2, 4 and 6 dpi. Thereafter, virus DNA was extracted and amplified by qPCR.

Cytokine Detection in Adenovirus-Infected 3D-Cultured 293T Cells

3D-cultured 293T cells seeded in 96-well plates were infected with adenovirus as previously described. The supernatant was collected at 6, 12 hpi and 1–10 dpi and stored at -80°C . The control group was set up without infection. TNF- α and IL-8 secretion levels were detected by ELISA. Cytokine levels in samples were calculated according to a standard curve.

Statistics

All statistical analyses were performed using GraphPad Prism 5.0 unless otherwise noted. Each data point represents the mean of three independent experiments at least. Data are represented as the mean value \pm SD of a triplicate experiment.

Results

Characterization of the RADA16-I Peptide Scaffold

The nanoself-assembling peptide RADA16-I is a kind of biomaterial formed by the self-assembly interaction of molecules. It has a unique amino acid sequence (Ac-RADARADARADARADA-CONH₂) and can self-assemble into a nanofiber hydrogel with a water content of

more than 99% in physiological saline solution;^{22,23} its fibre mesh aperture is approximately 5–200 nm.²⁴ SEM (Figure 1A) and TEM (Figure 1B) showed that RADA16-I self-assembled into highly crosslinked nanofibers with uniform width and height; the fibres were uniformly long and entangled with each other to form a 3D network. These data suggested that the RADA16-I hydrogel was similar to the ECM structure that makes up the microenvironment in vivo.

Characterization of 3D Cultures of 293T Cell Lines

The sequence RAD (arginine-alanine-aspartate) in the RADA16-I and the sequence RGD (arginine-glycine-aspartate) in the integrin ligand-binding site on the cell membrane are similar,²⁵ which can provide a 3D culture environment similar to natural ECM for cell adhesion and growth. In this study, cell proliferation of 293T cells cultured in the RADA16-I hydrogel that began as single cells and gradually formed dense multicellular spheroids and grew well was visible at 3 d, peaked at 11–15 d, and then gradually declined (Figures 2 and 3A). To further check the cell viability in the RADA16-I hydrogel, we stained cells with Ca-AM (green), which is hydrolysed to a green fluorescent product in living cells, and PI (red), which is able to pass through only the compromised membranes of dead cells (Figure 3B). Large green fluorescent areas were observed, and the green fluorescence was still visible in the cell mass after 30 days of culture (Figure 3C), suggesting that cells cultured in the RADA16-I hydrogel maintained good viability. Compared with 3D

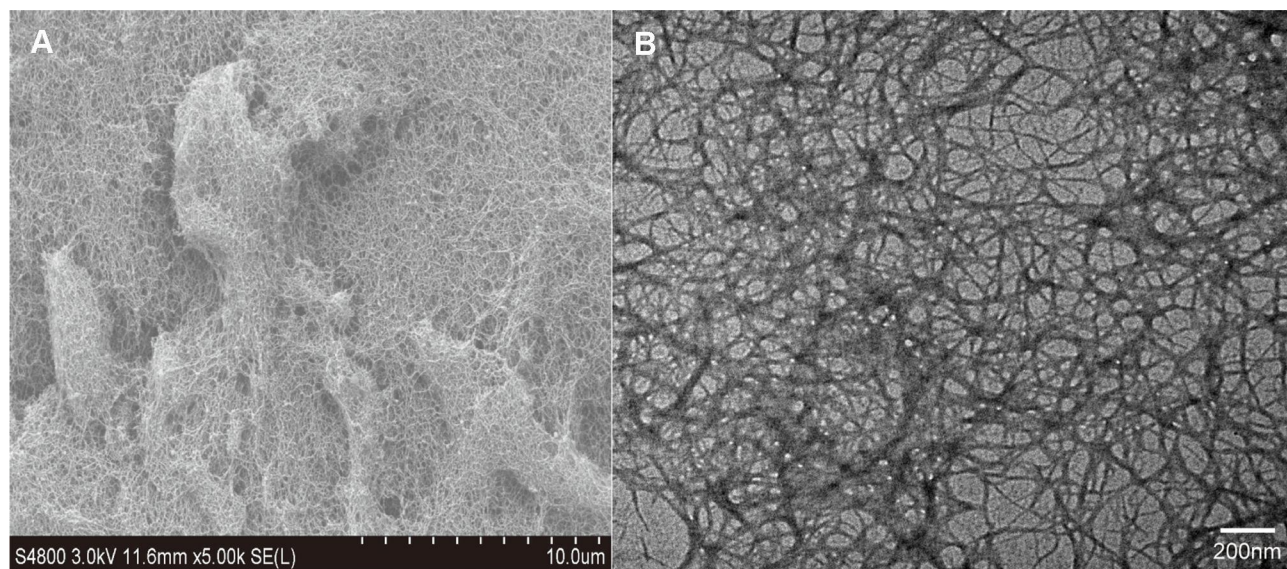


Figure 1 RADA16-I was evaluated by SEM and TEM and applied to 3D cell cultures. **(A)** SEM image of RADA16-I peptide scaffold nanofibers. **(B)** TEM image of RADA16-I peptide scaffold nanofibers.

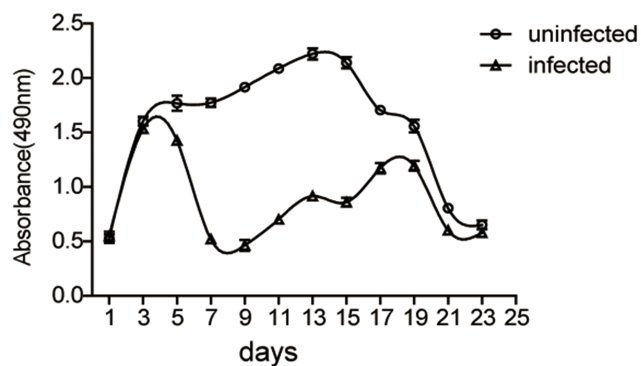


Figure 2 Proliferation of 293T cells in 3D culture by MTS.

culture, there is a main limitation associated with a traditional 2D culture that cells were grown on flat dishes optimized for cell attachment and growth (Figure 3A). Even if the serum concentration is reduced to 2%, the culture time can only be appropriately prolonged, but the cells no longer grow. Therefore, 3D culture that can form multicellular spheroids and maintain cell proliferation for a long time is more conducive to study the long-term virus culture in vitro and explore the relationship between viruses and hosts.

Viral Proliferation in 3D Culture

To analyze whether 3D-cultured 293T cells were susceptible to adenovirus infection, 293T cells in 2D culture and 3D culture were infected with adenovirus-EGFP. As shown in Figures 4A and 5A, the green fluorescence of cells in 2D culture was visible at 24 hpi, and the fluorescence intensity was strongest at 48–72 hpi. In addition, apparent cytopathic effect (CPE) could be observed, and the cells gradually became necrotic and detached at 4–6 dpi. By contrast, green fluorescent cell spheroids in 3D culture were observed at 60–72 hpi, but the fluorescence intensity was very weak. Thereafter, the number of fluorescent cells and fluorescence intensity increased gradually from the edge of the gel, peaking on 8 dpi, then decreasing gradually and peaking on 16 dpi and 22 dpi, respectively (Figures 4A and 5). As shown in Figure 4B vesicles and depressions of multicellular spheroids were visible at 5 dpi in 3D culture, and cytolysis was observed at 9–12 dpi, at which point individual cells were visible. With the extension of the incubation time, the number of dead cells in the RADA16-I hydrogel gradually increased, and fragmentation and cytolysis of cell spheroids were visibly observed at 15 dpi (Figure 4B). To further analyze the difference between the cytopathic effects caused by adenovirus proliferation in the 3D culture and those in the traditional 2D culture, infected-293T cells from these two culture systems were examined by a TEM assay. In the 3D

culture, we observed significant cytopathic effects such as swelling of the mitochondria and expansion of the endoplasmic reticulum at the 9th and 15th dpi. Furthermore, a small number of virions with diameters of 60–90 nm were significantly observed in the cytoplasm of 3D-cultured 293T cells (Figure 6A). In addition to swelling of the mitochondria and expansion of the endoplasmic reticulum, the formation of autophagosomes and a large number of autophagic lysosomes were observed at 3 dpi in the 2D culture system. Furthermore, we found that most of the virions with diameters of 60–90 nm were located in autophagic lysosomes, and the number of virions was more than that in 3D culture (Figure 6B). The viability of 3D-cultured 293T cells was affected by adenovirus infection. Therefore, an MTS assay was used to evaluate the viability of 293T cells after infection. As shown in Figure 2, the cells' viability appeared to be decreased at 3 dpi, reaching its lowest point at 7–9 dpi, then increasing gradually to the 13th dpi and decreasing gradually, reaching its lowest point at 15 and 22 dpi. Furthermore, we next monitored viral DNA at different time points post-infection by qPCR. The results showed that multiple peaks appeared in adenovirus DNA levels, and their proliferation characteristics were consistent with the results of the EGFP assay (Figure 7A). We also included controls without cells in 3D conditions. Also shown in Figure 7A, adenovirus DNA was detected only when 293T cells were present, demonstrating that the signal obtained was not due to the amplification of material-bound input adenovirus DNA. These results indicated that adenovirus could replicate and proliferate continuously in the 3D culture system with the nanoself-assembling peptide RADA16-I as scaffolds and could cause cytopathic effects that were not exactly the same as those cultured in the 2D system.

Characterization of Adenovirus Produced by 3D-Cultured Cells

We then investigated the infectivity of virus particles released from adenovirus-infected spheroids. We found that supernatants derived from adenovirus-infected 293T multicellular spheroids were infective, and their infectivity was not significantly different from that of 2D culture (Figure 7B). When cells are infected by viruses, to resist viral invasion, the interaction with viruses can stimulate cells to secrete cytokines such as TNF- α , IP-10, IL-6, and IL-8 to activate an immune response.^{26,27} To further confirm whether 3D cultures were infected by adenovirus and whether virus proliferation has effects on host cells, TNF- α and IL-8 in the media of the 3D cultures were examined by ELISA at the indicated time points

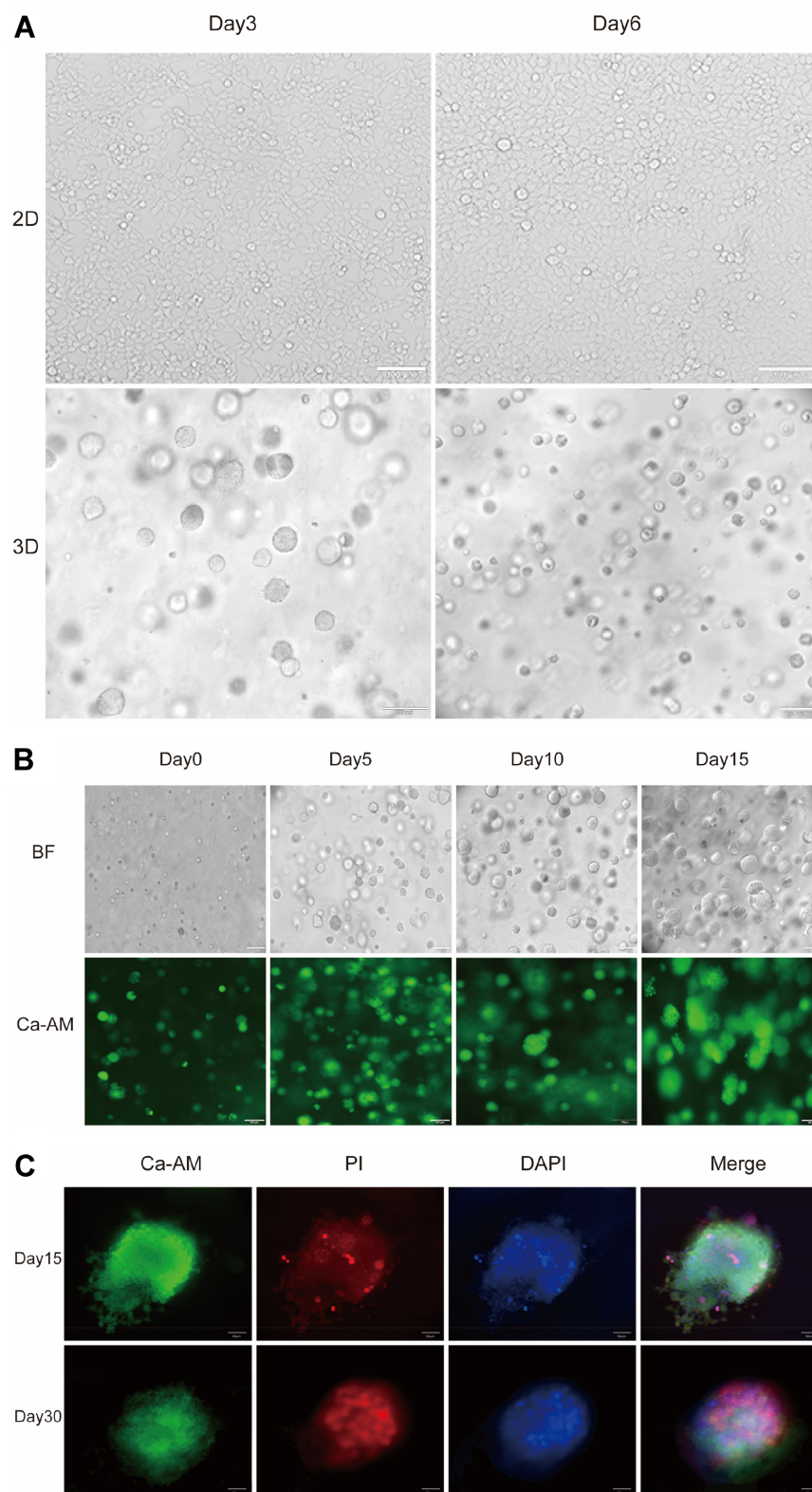


Figure 3 Growth and morphological characteristics of 293T cells in 3D culture. **(A)** Phase-contrast images of 293T cells cultured in 2D and 3D conditions. In 2D culture, the cells grew in a flat monolayer and gradually increasingly overlapped; in 3D culture, individual 293T cells formed dense multicellular spheroids. Bar, 100 μm . **(B)** Morphological characteristics and cellular viability of the 3D spheroids after 0, 5, 10, and 15 days of cultivation. In the 3D system, with the extending of cultivation time, the 3D multicellular spheroids gradually increased in size. The surface of the multicellular spheroids kept smooth in the early stage of culturing, but gradually, more and more bulges appeared. **(C)** Fluorescent staining of 3D multicellular spheroids after 15 and 30 days of cultivation. Ca-AM staining in green shows the live cells, PI staining in red shows the dead cells, and DAPI staining in blue shows the cell nuclei. Dead cells increased in number with the extension of cultivation time, but the viability of the cells was still high at 30 days. Bar, 50 μm .

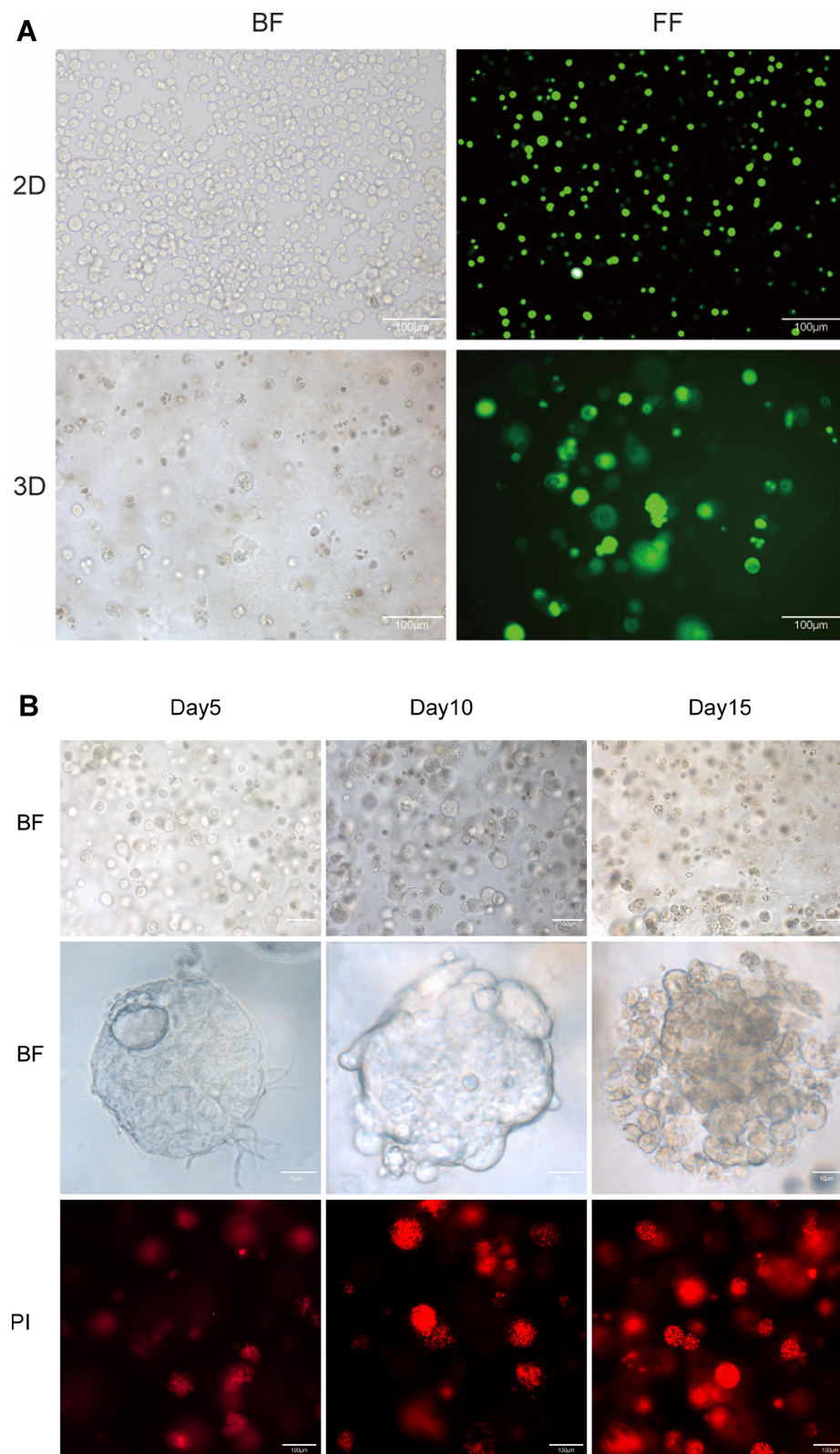


Figure 4 Growth and morphological characters of 3D-cultured 293T cells after adenovirus infection. **(A)** Phase-contrast image and fluorescence microscope image of 2D and 3D 293T cultures at 3 dpi and 10 dpi, respectively; **(B)** The morphological characteristics of 3D-cultured 293T cells at 5 dpi, 10 dpi, and 15 dpi. With the extension of culture time, the cell mass appeared as depressions, vesicles and dissolution. The dead cells (PI staining in red shows that) gradually increased. Bars, 100 μm (top and bottom) and 10 μm (middle).

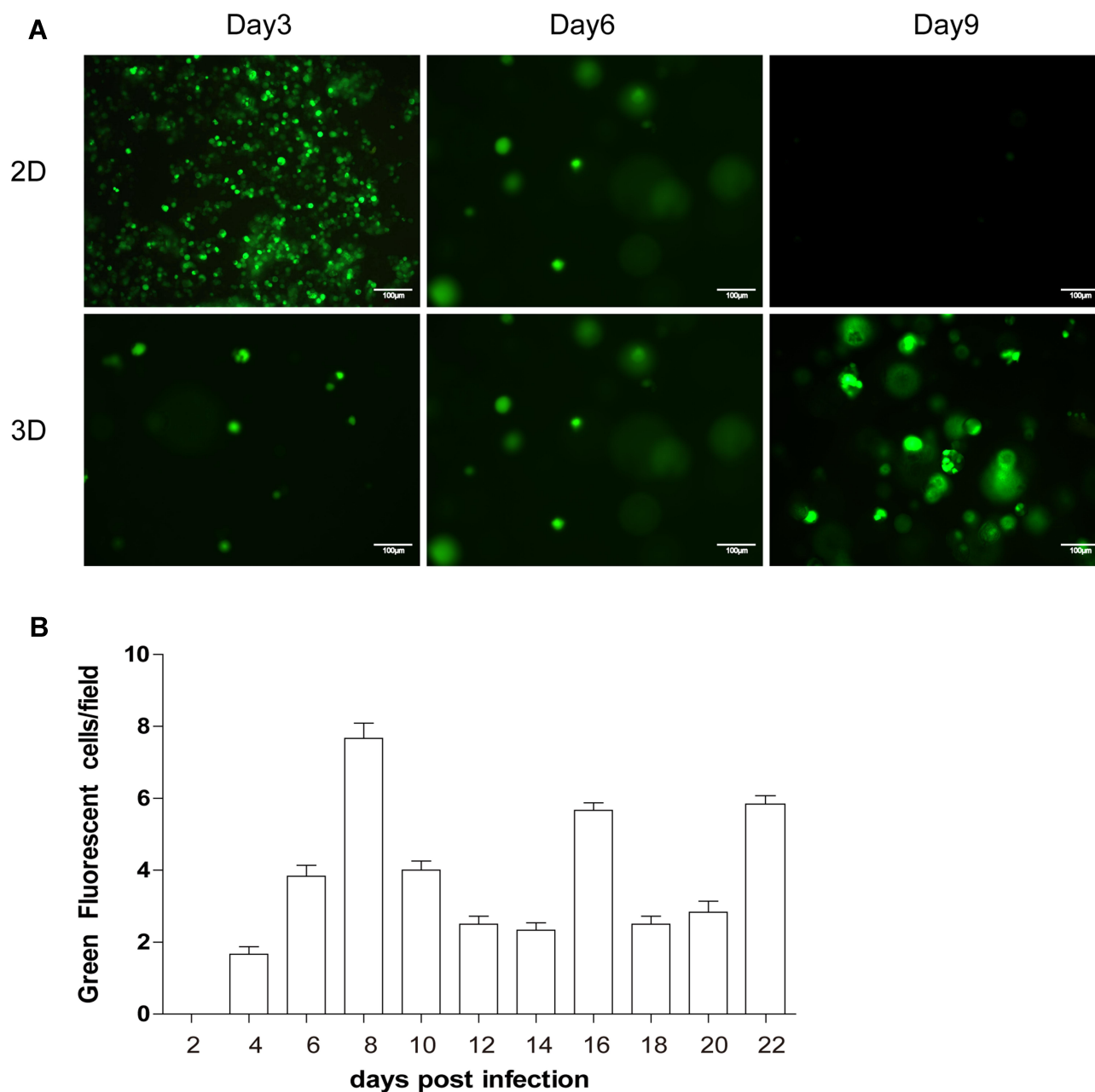


Figure 5 Proliferation of 293T cell in 3D culture after adenovirus infection. **(A)** Fluorescence microscope observation of 2D and 3D-cultured 293T cells at 3 dpi, 6 dpi and 9dpi. Bars, 100 µm; **(B)** Fluorescence counting in 3D-cultured 293T cells at different time after adenovirus infection.

after infection with adenovirus. As shown in [Figure 8](#), the cytokine increases appeared as multiple peaks in the 3D cultures after infection with adenovirus, but the expression levels of IL-8 and TNF- α as well as the secretion trends were different in the cell cultures. Compared to the uninfected cell cultures, expression of IL-8 increased significantly at 3 dpi, reaching three peaks at 4 dpi, 7 dpi and 10 dpi ([Figure 8A](#)). The expression of TNF- α increased less in the early stage of infection than in the uninfected cell cultures, the first peak appeared at 3 dpi, then the level of TNF- α increased significantly at 7 dpi

and reached its another peak at 9 dpi ([Figure 8B](#)). The above data suggested that the virions produced by the 3D cultures were still infectious, and virus proliferation had effects on 3D-cultured 293T cells. In a word, this 3D culture model can be applied to isolation and cultivation of adenovirus in vitro.

Discussion

The nanoself-assembling peptide RADA16-I as a good 3D scaffold has been successfully used in 3D culture for

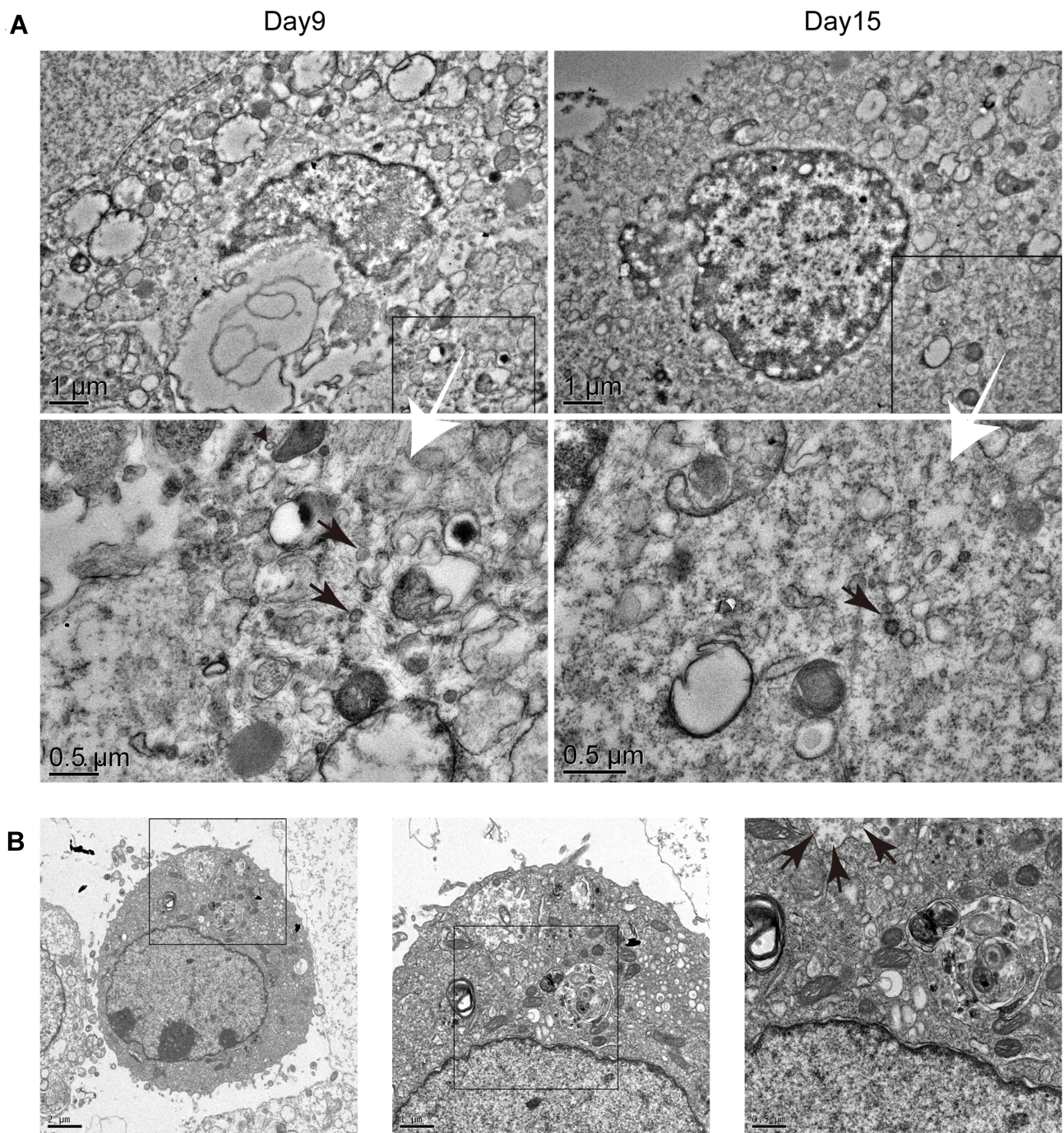


Figure 6 TEM analysis of adenovirus virions in 3D culture. **(A)** Suspected virions were immediately detected in 3D-cultured 293T cells at the 9th and 15th dpi. **(B)** Observation of adenovirus particles by TEM three days after adenovirus infection in 2D-cultured 293T cells. The box area was partially enlarged, and the virus-like particles are shown in the black arrow.

different cells^{20,21} because it can be chemically synthesized and has several advantages over other traditional 3D culture materials (such as Collagen and Matrigel) including that it is a single-ingredient with high purity, is non-immunogenic, and is pathogen-free. As an ionic hydrogel, RADA16-I can be self-assembled to form a 3D-nanofibre mesh structure under ionic conditions with porosity that is

similar to that of natural ECM²⁸ with high moisture, which is beneficial for cell adhesion, growth, diffusion and absorption of various nutrients and signal molecules in the microenvironment.^{29,30} Moreover, it is easier to operate than Collagen or Matrigel. Our study found that 293T cells in RADA16-I hydrogel can maintain cell proliferation for a long time, which provides a possibility to study

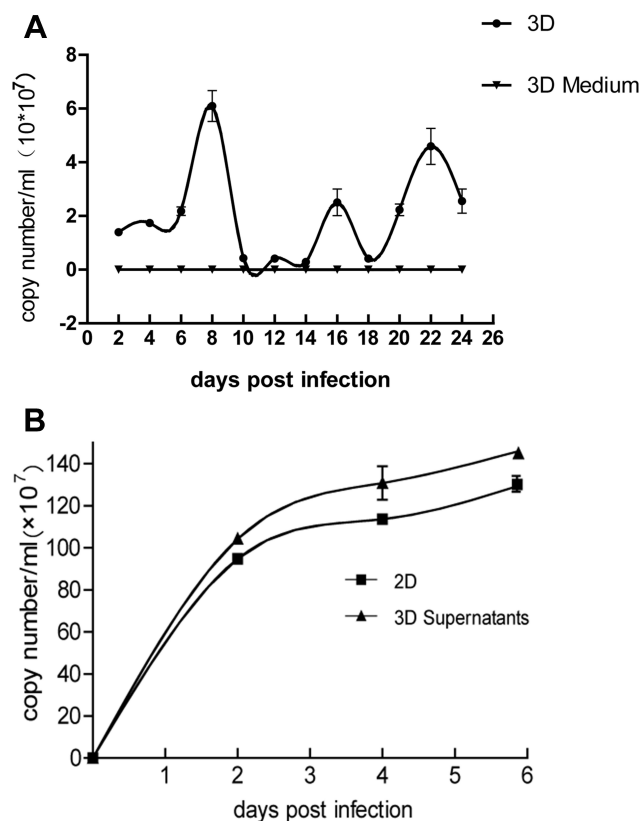


Figure 7 qPCR measurement of adenovirus proliferation. **(A)** Adenovirus proliferation in 3D culture was detected by qPCR. Three-dimensional adenovirus amplification in 3D-cultured 293T cells; the 3D medium indicated adenovirus amplification in RADA16-I hydrogel without 293T cells. **(B)** Infectivity of 3D-amplified virions was measured by qPCR. Two-dimensional standard adenovirus amplification in 2D culture; 3D supernatants indicated 3D-generated adenovirus virions (1×10^7 copies based on the PCR amplification quantification) amplification in 2D culture. Three-dimensional-generated adenovirus virions were included in the 3D culture supernatants, which were harvested on the 6th day after infection.

long-term virus culture in vitro and explore the relationship between viruses and hosts.

The diameter of the fibre mesh formed by the nano-self-assembling peptide RADA16-I is approximately 5–200 nm.²⁴ Therefore, adenovirus with a diameter of approximately 60–90 nm could infect 293T cells through the scaffold pores of RADA16-I; this was confirmed in our study that the virus particles in 293T cells cultured in RADA16-I were observed under TEM at 9 and 15 dpi. In vivo, the virus infects susceptible cells and releases a large number of progeny virus that will infect the surrounding cells again, causing the CPEs and corresponding clinical symptoms in the body. However, in vitro, the cells cultured in the 2D system were a monolayer in-plane. After inoculation of virus, all the cells in plane growth could contact the virus in a short time, CPEs appeared soon, and the cells gradually became round and necrotic and fell off. Therefore,

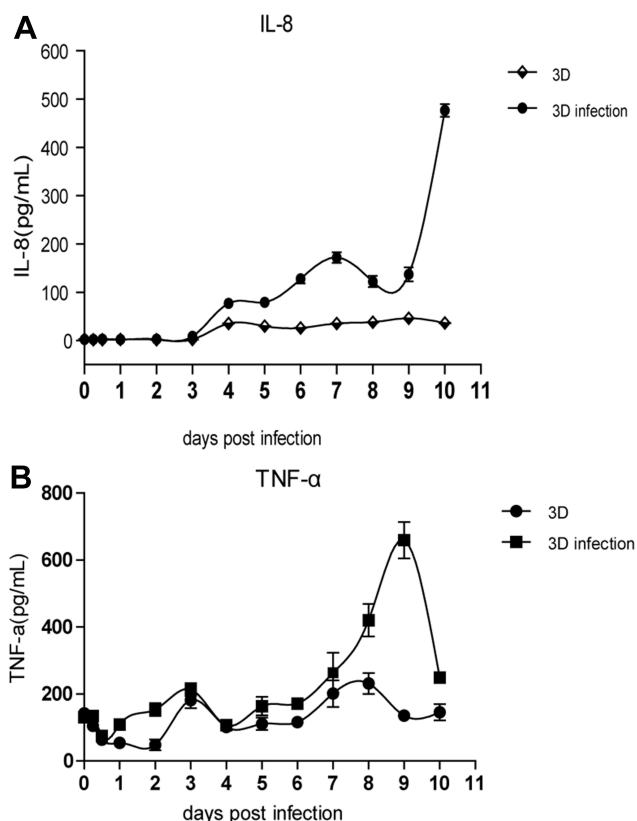


Figure 8 Cytokine secretion measured in the 3D-cultured 293T cells after infection with adenovirus. TNF- α **(A)**, IL-8 **(B)** levels in the media of 293T cultures at the indicated day after infection were measured by an ELISA assay.

2D culture is not suitable for the study of long-term infection. In the 3D cell culture system, by contrast, the virus adsorbs on the surface of the multicellular spheroids and first infects the outermost cells. After replication and proliferation in these cells, a large number of progeny viruses are released to infect the central cells or adjacent multicellular spheroids, while the uninfected cells continue to divide and grow. Therefore, our study found that the period of virus culture in RADA16-I was much longer than that in the 2D culture, and there were multiple proliferation peaks, which was similar to the reports of Berto et al.^{18,31} This characteristic of proliferation may be related to the interaction between the virus and the host (ie, infection, release, reinfection), which may also be the cause of multiple secretion peaks of the cytokines TNF- α and IL-8 after virus infection in the 3D culture, which is similar to Chen et al's¹⁸ findings in the study of a 3D culture of HBoV and HRV-C. Taken together, compared with a 2D cell culture, a 3D cell culture can simulate the process of virus infection in vivo.

In this study, RADA16-I was used as a scaffold to construct a 3D cell culture model of adenovirus in vitro, but there are still some limitations. First, adenovirus can proliferate in the RADA16-I 3D culture system for a long time, but there is no further study on the infection mechanism because the virus is an empty vector. Moreover, whether other viruses can also be cultured in the 3D culture system for long-term culture remains to be verified. Second, with the continuous development of 3D cell culture technology, researchers clearly recognized that the mechanical properties (such as material hardness and pore structure) and biochemical properties (such as biocompatibility and degradability) of scaffold materials may have an important impact on cell viability, adhesion, differentiation and migration.³² Although the nanoself-assembling peptide RADA16-I is a type of good 3D culture scaffold material, it still has problems such as insufficient mechanical strength and low shear resistance.³³ It is uncertain whether the nanoself-assembling peptide RADA16-I is the best 3D scaffold material at present, but it is clear that any in vitro 3D culture system cannot completely and accurately simulate the human microenvironment because the organism is a complex environment in which many cells, organs and tissues coexist. Moreover, the process of the virus infecting the organism, replicating and proliferating in cells, and causing a series of pathological changes is a complex pathophysiological process in which many cells and tissues participate. Therefore, if RADA16-I can be used to construct a 3D co-culture model of various cells, it will be important to study the pathogenic mechanism of viruses and explore the interactions between viruses and hosts.

Conclusion

To date, 3D cell culture is considered to be a better cell model in vitro. However, 3D culture scaffolds have different materials, which may have some problems such as being immunogens or pathogens, having too large pores, and being difficult to obtain and shape. Most importantly, it is difficult to standardize and obtain accurate results. Our research showed that the nanoself-assembling peptide RADA16-I can be used as scaffolds for 3D cell culture of adenovirus. Combined with the characteristics of adenovirus vectors such as high transgenic efficiency, high titres regardless of the state of cell division, and easy evaluation for biological viability, we hope that this 3D culture method of adenovirus can be used not only in the study of viral pathogenesis but also in the study of antiviral drugs, vaccines, viral gene therapy and other fields in the future.

Abbreviations

3D, three-dimensional; 2D, two-dimensional; ECM, extra-cellular matrix; TEM, transmission electron microscope; SEM, scanning electron microscope; DMEM, dulbecco's modified eagle medium; Ca-Am, calcein-AM; dpi, days post infection; EGFP, enhanced green fluorescent protein; qPCR, real-time fluorescent quantitative PCR; ELISA, enzyme-linked immunosorbent assay; hpi, hours post infection; CPE, cytopathic effect.

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Disclosure

Di-Shu Ao and Lu-Yao Gao should be regarded as co-first authors. The authors declare no conflict of interest.

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