# 哺乳动物细胞悬浮培养技术及其应用

陈铭杰[1] 毕海洋[1] 马懿昭[1] 章圣华[1] 张艳艳[1] 张燕茹[1] 杨松柏[1] 周晓龙[1]
乔自林[2] 李向臣[1] 武永淑[1]*

([1]浙江农林大学动物科技学院•动物医学院, 浙江省畜禽绿色生态健康养殖应用技术研究重点实验室, 动物健康互联网检测技术浙江省工程实验室, 动物医学与健康管理浙江省国际科技合作基地, 中澳动物健康大数据分析联合实验室, 杭州 311300; [2]西北民族大学, 甘肃省动物细胞技术创新中心, 兰州 730030)

**摘要** 悬浮培养技术是将细胞悬浮于培养液中, 用于病毒增殖或蛋白表达的技术。该技术具有细胞培养规模大、目的产物表达量高、表达产物质量好和利于产业化等优点。当前, 多种细胞已通过选择合适的生物反应器、使用微载体和无血清培养基等方法被成功悬浮培养, 用于病毒灭活疫苗、重组蛋白和细胞治疗等领域。该文阐述了哺乳动物细胞悬浮培养技术以及其在病毒灭活疫苗、重组蛋白和细胞治疗等方面的应用, 以期为悬浮培养技术的发展和应用提供一定参考。

**关键词** 哺乳动物细胞; 生物反应器; 微载体; 无血清培养基; 生物制品

# Suspension Culture Technology of Mammalian Cells and Its Application

CHEN Mingjie[1], BI Haiyang[1], MA Yizhao[1], ZHANG Shenghua[1], ZHANG Yanyan[1], ZHANG Yanru[1], YANG Songbai[1], ZHOU Xiaolong[1], QIAO Zilin[2], LI Xiangchen[1], WU Yongshu[1]*

([1]*Key Laboratory of Applied Technology on Green-Eco-Healthy Animal Husbandry of Zhejiang Province, Zhejiang Provincial Engineering Laboratory for Animal Health Inspection & Internet Technology, Zhejiang International Science and Technology Cooperation Base for Veterinary Medicine and Health Management, China-Australia Joint Laboratory for Animal Health Big Data Analytics, College of Animal Science and Technology & College of Veterinary Medicine of Zhejiang A&F University, Hangzhou 311300, China*; [2]*Gansu Technology Innovation Center of Animal Cell, Northwest Minzu University, Lanzhou 730030, China*)

**Abstract** Suspension culture technology involves suspending cells in a culture medium, which is utilized for virus propagation or protein expression. This technology is often utilized for purposes such as virus proliferation or protein expression. It has several advantages, including scalability for scale-large cell culture, high expression levels of target products, high quality of products and advantage for industrial application. Various cell types have been successfully adapted to suspension cultures through the use of suitable bioreactors, microcarriers, and serum-free media. These cells are currently being utilized in a range of applications, including inactivated virus vaccine production, recombinant proteins and cell therapy. This review provides an in-depth analysis of mammalian cell suspension culture domestication technology and its applications in the fields of inactivated virus vaccines, recombinant proteins, and cell therapy. By providing insights into the development and application of suspension culture technology, this review aims to serve as a valuable resource for researchers in this field.

**Keywords** mammalian cells; bioreactor optimization; microcarrier; serum-free medium; biological products

收稿日期: 2024-05-15 接受日期: 2024-08-22
西北民族大学生物医学研究中心开放基金(批准号: BRC-KF202304)资助的课题
*通信作者。Tel: 18193177285, E-mail: wuyongshu0224@zafu.edu.cn
Received: May 15, 2024 Accepted: August 22, 2024
This work was supported by the Open Fund of the Biomedical Research Center, Northwest Minzu University (Grant No.BRC-KF202304)
*Corresponding author. Tel: +86-18193177285, E-mail: wuyongshu0224@zafu.edu.cn

自1885年ROUX[1]用温生理盐水培育鸡胚组织开始，随着细胞培养技术的不断发展，多种用于科研和生产的细胞系被不断开发。如非洲绿猴肾细胞(Verda reno, Vero)、中国仓鼠卵巢细胞(Chinese hamster ovary cell, CHO)、人胚胎肾细胞293(human embryonic kidney 293, HEK293)等动物全悬浮细胞系，已大规模应用于疫苗和重组蛋白等的生产。

细胞系有贴壁培养和悬浮培养方式。贴壁培养作为传统的细胞培养方式，便于观察和实验操作，但是得到的细胞数量有限、操作繁琐。随着技术发展，微载体悬浮培养应运而生，可扩大培养体系，节约空间。然而，微载体多为一次性使用，价格昂贵，需要添加血清以支持细胞生长。此外，这项技术相对复杂且不太完善，培养规模具有一定限制性[2]。近年来，全悬浮培养技术兴起，相较于贴壁培养与微载体悬浮培养，全悬浮培养逐步降低了血清浓度、血清生产批次差异以及潜在的感染风险，从而降低了下游纯化难度。由于不需要贴附基质，且在全悬浮培养体系中细胞大多呈现单个细胞悬浮状态，故全悬浮培养方式能够增加细胞与培养基及溶解氧的接触面积，提高细胞密度，节约设备空间，提高设备利用率，便于规模化生产，可适用于细胞、病毒灭活疫苗疫苗和重组蛋白等大量制备。因此，细胞悬浮培养技术未来应用场景将越发广泛。

## 1 细胞悬浮培养技术

贴壁细胞通过特定驯化过程转变为能在悬浮状态下生长的细胞，能够实现更高效的生物反应器操作和生产流程。生物反应器因其规模化培养而成为悬浮培养技术的首选，目前许多悬浮细胞系通过生物反应器生产目的产物，然而，在细胞悬浮驯化过程中，发生失巢凋亡可能会导致其状态恶化甚至死亡，通过组学分析和基因编辑调控相关基因的表达，有利于进一步促进细胞悬浮生长。为了提高细胞密度，学者们从生物反应器设计、培养参数等方面进行优化。微载体悬浮培养是细胞附着在微载体上置于生物反应器中悬浮培养的技术。无血清悬浮培养基的开发，能够降低生产成本并提高产品的一致性。

### 1.1 生物反应器

生物反应器是一种用于培养微生物、悬浮细胞和组织等生物体的设备。生物反应器具有培养规模大、批次差异小、易于调控、智能化和管道化等优点，被广泛用于各种悬浮细胞的大规模培养，以提高目的产物的表达量。当前用于哺乳动物细胞悬浮培养的生物反应器技术较为成熟，包括连续培养、灌流式培养和激流式培养等，表1对不同类型生物反应器培养方式及优缺点进行了总结。

有研究从流体力学角度设计生物反应器，旨在降低剪切力对细胞的损伤。生物反应器细胞培养液通常呈非牛顿流体特性。然而，目前许多生物反应器的设计中，流体动力学的模拟仍基于牛顿流体假设，这可能导致剪切应力量化不准确，影响细胞生长和分化。而波浪式生物反应器、激流式生物反应器、中空纤维生物反应器等生物反应器的开发，有效缓解了剪切力对细胞的损伤[5,7-8]。

目前生物反应器中很多基于膜系统的细胞分离技术是高效的，但是存在堵塞等导致高成本的问题。KWON等[9]利用惯性微流体学原理设计并制造了一种高效微流控细胞分离装置。该装置基于惯性和螺旋通道产生的附加阻力，有效地从生物反应器中连续去除了小尺寸的非活性细胞和碎片，提高了细胞培养的质量和生物制品的生产效率。SYED等[10]设计了一个两步微流控系统，首先使用微型水力旋流器分离细胞，再结合多重螺旋微通道进一步提高分离效率，即使在高达8 000万个/mL的细胞浓度下，依然可达到超过90%的细胞回收率，有效解决了生物反应器中高密度细胞的连续保留问题。

除了生物反应器设计对细胞培养的影响外，优化细胞培养参数也是生物反应器培养考虑的关键因素。WIEGMANN等[11]使用由Applikon公司开发的单独控制pH值、溶解氧和温度，并支持在线自动添加培养液的一款微型生物反应器，分批次悬浮培养了CHO细胞。代为俊等[12]发现在1 500 L反应器中，60 r/min的转速和22.5 L/min的深层通气速率能够较好满足犬肾上皮(Madin-Darby canine kidney, MDCK)细胞培养需求和提高$CO_2$的移除效果，同时避免剪切力对细胞的损伤。杨惠清等[13]通过效应面法预测了用于培养Vero悬浮细胞的最佳参数，最终将细胞密度提高了0.28倍，实现了规模更大的高密度培养。综上，培养参数是细胞培养的关键因素，而优化培养参数是提高细胞密度的重要手段。

失巢凋亡是细胞与细胞外基质和其他细胞失去接触而诱导的一种特殊的程序化细胞死亡形式。在悬浮培养过程中，贴壁细胞状态不佳与失巢凋亡

表1 生物反应器类型

Table 1 Types of bioreactors

| 生物反应器类型 Types of bioreactors | 培养方式 Culture method | 优点 Advantage | 缺点 Disadvantage | 参考文献 Reference |
|---|---|---|---|---|
| Stirred tank bioreactor | Using the external stirring power method, the liquid will be driven to stir, to achieve the cell culture in suspension conditions | Easy to operate and monitor, suitable for mass production | Shear forces have a large impact on cells | [3] |
| Airlift bioreactor | Utilizing gas vapor, in conjunction with bioreactor operation, the vapor force of the gas mixture is used to cultivate cells | Strong resistance to external microbial contamination and good airtightness | In high-density culture, the cells are not mixed uniformly enough, plus the use of gas as a power source makes the operation more complicated | [4] |
| Wave bioreactor | Mixing and oxygen dissolution by means of wave oscillations | Protected from shear damage | Scaling up is harder and more costly | [5] |
| Perfusion bioreactor | Replenish nutrients consumed by cells during growth and production and excrete metabolic wastes | Continuous supply of nutrients and removal of wastes to maintain a stable culture environment | Complex equipment operation, high maintenance costs, high media quality requirements | [6] |
| Turbulent bioreactor | Generates turbulent dissolved oxygen molecules through mechanical oscillation, without the use of stirring or aeration methods | Protected from shear damage | Single-use, low material durability, limited volume, not suitable for mass culture | [7] |
| Hollow fiber bioreactor | Hollow fibre membrane technology is used to achieve the exchange of material between the cells and the culture medium through capillary-like cartridges. Cells are cultured inside the fibres, while the micro hollow tubes are responsible for transporting nutrients and removing metabolic waste | Prevents shear damage to cells, efficient substance exchange, suitable for a wide range of cells | Complexity of equipment operation, high equipment and maintenance costs, cellular and metabolic wastes may settle on the membrane leading to membrane clogging | [8] |

有关[14]。DAI等[15]证明敲除*PABPC1*基因后细胞获得了悬浮培养特征, 从而降低了细胞凋亡。HARJUNPÄÄ等[16]通过研究细胞黏附分子在肿瘤免疫微环境中的作用, 发现整合素αVβ3通过抑制p53和caspase-9增强了肿瘤细胞的抗凋亡能力, 同时整合素αVβ5和αVβ3还上调了抗凋亡蛋白Bcl-2和Bcl-XL的表达。MACDONALD等[17]发现, 敲除*Bax*和*Bak1*可延迟并减慢CHO细胞凋亡。另外, TANG等[18]发现敲除*Bax*可提高细胞活力。抑制细胞失巢凋亡的策略已被证实能有效提升细胞的活力。

细胞黏附性对其形态、功能和生存至关重要。在悬浮培养条件下, 细胞需要适应缺乏传统黏附基质的环境, 这种适应性变化涉及多种分子和信号通路的调控。PECH等[19]发现MDCK细胞在悬浮培养条件下, 钙黏蛋白-1和小窝蛋白-1表达量的降低, 以及AMPK表达量的增加, 共同影响细胞黏附、代谢和自噬, 从而使其能够适应悬浮生长环境。SÈNE等[20]发现悬浮Vero细胞表现出上皮–间充质转化途径的抑制, 以及细胞黏附分子、细胞骨架和细胞外途径的激活。此外, 糖酵解途径的抑制与天冬酰胺代谢途径的激活相平衡, 促进了细胞对营养剥夺的适应。但黏附连接蛋白的表达量下调和*FYN*基因的表达量降低可能导致细胞活力低下和倍增时间长。CHU等[21]的研究显示, *siat7e*基因的高表达水平

与MDCK细胞增强的悬浮生长能力相关，这可能是通过促进上皮–间充质转化途径中受体酪氨酸激酶c-Met的自分泌激活和减少细胞黏附来实现的。因此，调控细胞黏附相关基因表达或许是促进细胞悬浮驯化的可行思路。

综上所述，生物反应器是大规模培养悬浮细胞的主要载体，也是目前培养悬浮细胞的主要方式。因其具有规模化、易控制、智能化和管道化等优点而被广泛应用。此外，科学设计生物反应器、优化培养参数、减少细胞凋亡以及控制细胞黏附等一直是生物反应器悬浮培养技术的主攻研究方向。

### 1.2 微载体悬浮培养

微载体悬浮培养是将细胞黏附在微载体表面，在培养液中进行培养的技术，其本质依然是贴壁培养。然而，与传统贴壁培养相比，微载体悬浮培养具备细胞与培养液接触面积大、培养环境易监控、占用空间小、操作性、可系统化和自动化等优点。该技术适用于难以悬浮驯化或悬浮后生长性能易变的细胞系。它可提高细胞密度和活力，有助于实现细胞的高效悬浮培养。然而，该技术也存在一定缺点，如价格高昂且使用血清导致的外源病原潜在污染等。另外，细胞与微载体之间的黏附和消化是最终影响细胞数量和质量的两个关键阶段，若细胞在微载体上附着效率低或分布不均，则会导致细胞产量低于预期。不适当的分离方式会降低细胞的恢复能力，对细胞存活产生不利影响。

随着科学技术和材料学的发展，微载体材料也快速发展。1967年，VAN WEZEL[22]将兔肾细胞等细胞附着在由天然葡聚糖聚合物制成的固体小珠上，成功培养成细胞系。目前已有多种微载体材料，如明胶、纤维素、聚苯乙烯和聚乙烯等，总结见表2。

近年来微载体悬浮培养技术虽在间充质干细胞、神经干细胞以及其他类型细胞的扩增和分化中应用广泛[29]。但其在材料选择、消化方法和传代策略等方面仍存在挑战，需要进一步解决[1]。

### 1.3 无血清培养基

经近百年的发展，培养基已经从天然培养基、合成培养基发展到无血清培养基和限定化学成分培养基。在无血清培养过程中，含血清替代成分培养基的选择很关键。无血清悬浮培养通过添加促红细胞生成素、神经生长因子、血小板裂解物和促有丝分裂因子等替代血清，能消除血清批次差异和病原体污染风险[30]。WU等[31]在CHO细胞的无血清适应过程中补充$Ca^{2+}$和$Mg^{2+}$显著降低了细胞倍增时间，

**表2 微载体材料特性**

**Table 2 Microcarrier material properties**

| 微载体材料<br>Microcarrier material | 特性<br>Features | 文献来源<br>Reference |
|---|---|---|
| Polysaccharides | Polysaccharides possess excellent biocompatibility and biodegradability, which typically facilitates cell adhesion and proliferation. They can be fabricated into microcarriers through various methods, such as emulsification and thermally induced phase separation techniques. However, the efficiency of cell detachment is relatively low, impacting the automation of the process and the associated costs | [23] |
| Protein-based microcarriers | Protein-based microcarriers possess high biocompatibility, controllable biodegradability, optimized porous structures to facilitate cell adhesion and proliferation, and tunable physicochemical properties to accommodate the cultivation requirements of various cell types | [24] |
| Synthetic polymer microcarriers | The material offers predictable degradation rates and controllable physicochemical properties. It can be fabricated into a porous structure through specific processes to enhance cell adhesion and diffusion. The robust structure facilitates fixation and support, making it suitable for high-density cell culture, although scaling up the process presents challenges | [25] |
| Magnetic microcarriers | Incorporating magnetic materials with biocompatible polymers, these constructs enable precise cell manipulation and tissue assembly under the guidance of an external magnetic field | [26] |
| Self-healing microgels | These certain microgels, such as PCB (polycarboxybetaine) microgels, possess self-healing capabilities and can rapidly reorganize through supramolecular interactions, providing a microenvironment akin to the extracellular matrix for cells | [27] |
| Thermo-responsive microgels | These microgels can alter their physical properties in response to temperature changes, facilitating thermo-responsive cellular reactions and drug release | [28] |

并提高了细胞密度和活力。JANG等[32]通过使用无血清Freestyle 293扩增培养基, 成功建立了HEK293细胞无血清培养的悬浮和贴壁细胞系, 细胞活力达90%以上。OGAWA等[33]为了扩大培养规模, 在悬浮培养条件下添加天然多糖FP001和FP003成功培养了人诱导多能干细胞来源的肠类器官。YANKAS-KAS等[34]通过使用Gibco™ OncoPro™ Tumoroid Culture Medium和特定组织补充因子, 实现了子宫内膜癌细胞长期稳定衍生和扩增, 同时保持了细胞对激素信号通路的依赖性。

目前已成功驯化出多个能够稳定遗传并且生产效率高的无血清全悬浮细胞系, 如幼年仓鼠肾(baby hamster kidney-21, BHK-21)、猪睾丸(swine testis, ST)、Vero等细胞系。希望未来开发出更多可促进细胞适应无血清培养的物质, 用于生物制品的高效稳定生产。

## 2 悬浮培养技术的应用

### 2.1 病毒灭活疫苗

自1962年CAPSTICK等[35]对BHK-21细胞驯化实现悬浮培养并用于兽用疫苗生产以来, 悬浮培养技术主要用于病毒灭活疫苗生产。病毒灭活疫苗主要是由病毒在敏感细胞系增殖后, 进一步灭活与佐剂乳化而成的生物制品。因此, 病毒大量繁殖和提高病毒滴度是疫苗生产的核心, 而悬浮培养细胞为病毒大量增殖提供了支持, 降低了工艺成本, 提高了产品质量和一致性, 使病毒灭活疫苗实现了质的飞跃[36]。

BISSINGER等[37]采用半灌流培养系统实现了MDCK悬浮细胞高密度培养, 用于高效生产甲型流感病毒。PARK等[38]成功建立了BHK-21悬浮细胞系并利用规模化生物反应器生产了口蹄疫病毒(foot-and-mouth disease virus, FMDV)。另外, 为了提高FMDV的产量, 他们开发了一种定制的无血清培养基, 该培养基包含特定组分以支持细胞的快速扩增。当培养规模从50 L扩大到200 L时, BHK-21悬浮细胞显示出与50 L生物反应器相当的细胞活力和FMDV滴度。目前, 我国FMD疫苗生产企业已完全实现悬浮培养BHK-21细胞生产FMD灭活疫苗, 并达到至少8 000 L的培养规模, 极大提高了FMDV的产量和质量, 实现了智能化、管线化和自动化的生产模式。WANG等[39]使用无血清ST503培养基悬浮驯化牛肾细胞, 使其成功用于规模化生产I型牛疱疹病毒疫苗。Vero细胞被誉为疫苗生产中最高产、最安全和应用最广的细胞, 主要用于狂犬病毒、脊髓灰质炎病毒、乙型脑炎病毒、伪狂犬病毒、猪流行性腹泻病毒和鸡新城疫病毒等灭活疫苗的生产[40-45]。

此外, 悬浮培养技术也用到其他疫苗生产。例如, ST细胞生产轮状病毒和伪狂犬病毒灭活疫苗[46-47], 猪肾细胞生产猪丁型冠状病毒、猪圆环病毒疫苗[48-49]。悬浮细胞系培养不同病毒用于生产灭活疫苗(表3)。综上所述, 悬浮培养技术在疫苗尤其在病毒灭活疫苗中得到广泛应用和快速发展, 在人类和动物疫病的防控中发挥了中流砥柱的作用, 相信在未来会有更多支持病毒增殖的细胞被用于疫苗生产中。

### 2.2 重组蛋白

近几十年来, 大肠杆菌等原核表达系统在科学研究中一直占据主导地位。然而, 有研究发现, 哺乳动物细胞可以弥补原核生物缺乏特定辅助因子、分子伴侣和翻译后修饰导致的功能丧失和蛋白错误折叠等缺陷[50]。当前, 重组蛋白需求量极大, 故生物反应器和悬浮细胞系为重组蛋白生产提供了支持。

HEK293细胞系被广泛用于生产重组蛋白。PULIX等[51]通过评估HEK293细胞系基因插入的基因组安

**表3 用于增殖病毒的悬浮细胞系**

**Table 3 Suspension cell lines for virus propagation**

| 悬浮细胞<br>Suspension cells | 病毒<br>Virus | 文献来源<br>References |
|---|---|---|
| MDCK (Madin-Darby canine kidney) cells | Influenza A virus | [37] |
| BHK-21 (baby hamster kidney-21) cells | Foot-and-mouth disease virus | [38] |
| MDBK (Madine-Darby bovine kidney) cells | Bovine herpesvirus-I | [39] |
| Vero (Verda reno) cells | Rabies virus, poliovirus, encephalitis B virus, pseudorabies virus, porcine epidemic diarrhea virus, Newcastle disease virus | [40-45] |
| ST (swine testis) cells | Rotavirus, pseudorabies virus | [46-47] |
| PK (porcine kidney) cells | Porcine delta coronavirus, porcine circovirus | [48-49] |

全港位点并设计开发新的工程化变体，实现重组抗体稳定和可重复表达。HACKER等[52]将含有编码R-Spondin1和Noggin两种蛋白的基因的质粒转染到HEK293悬浮细胞系中，实现这两种蛋白的异源表达并将其用于维持肠道干细胞培养。此外，HEK293还用于生产重组人可溶性T淋巴细胞免疫球蛋白黏蛋白分子3、活化蛋白C、凝血因子VIII、凝血因子IX和胰高血糖素样肽1等重组蛋白[50,52]。

CHO细胞具有类似于人源细胞的翻译后修饰功能，更适合用于制备人用重组蛋白。BORSI等[54]通过鉴定CHO-K1悬浮细胞和贴壁HEK293细胞系的高度可变基因以研究它们各自的变异来源，结果表明，CHO-K1的高度可变基因在多个关键生物过程(包括细胞有丝分裂、细胞迁移以及发育过程)中表现出显著富集。由此推测相较于贴壁HEK293细胞系，CHO-K1悬浮细胞可能更加适用于生产重组蛋白。HUANG等[55]采用CRISPR-Cas9技术将人血白蛋白基因整合到CHO细胞系特定的染色体上，通过悬浮培养持续产生人血白蛋白。MASUDA等[56]基于CHO细胞开发了能够高度生产IgG1的细胞系，且该细胞系倍增时间显著缩短。由此可见，经悬浮驯化的CHO细胞系在生物制药中具有广泛的应用前景，希望将来有更多通过CHO悬浮细胞系大规模生产的重组蛋白用于临床治疗，满足社会需求。

### 2.3 细胞治疗

多能干细胞，如胚胎干细胞、间充质干细胞和诱导多能干细胞，可用于细胞治疗以取代受损器官或组织来治疗难治性疾病和损伤[57]。通常多能干细胞具有高度贴壁依赖性，悬浮驯化较为困难。然而，也有部分悬浮培养的干细胞已经用于细胞治疗[58]。

近年来，多种干细胞被成功悬浮驯化并扩增。CUESTA-GOMEZ等[59]使用三维垂直轮生物反应器在5天内实现了近100倍的诱导多能干细胞扩增，揭示了一种高效且可更安全地应用于临床的细胞治疗生产新策略。ACKERMANN等[60]开发的骨髓细胞复合物方法，通过悬浮培养诱导多能干细胞分化为骨髓细胞，进而生成巨噬细胞，有效解决了功能性巨噬细胞高效生产问题，对巨噬细胞研究和细胞治疗具有重要意义。BRAAM等[61]采用悬浮培养技术，成功将多能干细胞衍生的胰腺前体细胞进一步诱导分化为内分泌细胞，特别是表达胰岛素的β细胞，提高了细胞的功能性，使其能够响应葡萄糖刺激并分泌胰岛素，为糖尿病提供了一种潜在的治疗策略。SOUGAWA等[62]通过搅拌式悬浮生物反应器高效扩增诱导多能干细胞衍生的心肌细胞，用于治疗心力衰竭。随着重编程干细胞底层技术的突破，现在可使用化学小分子将人成体细胞诱导为多能干细胞，与体细胞核移植以及过表达转录因子调控细胞命运的方法相比，其调控性更强、操作更简便灵活，相信未来将会有更多通过化学物质诱导形成的干细胞用于细胞治疗[63]。

## 3 小结与展望

使用生物反应器、微载体以及无血清培养基等具备规模化、智能化和管道化等优点的细胞悬浮培养技术悬浮培养哺乳动物细胞，实现了大规模生产病毒灭活疫苗、重组蛋白以及用于疾病预防和治疗的细胞。然而，悬浮培养技术仍然面临许多挑战。首先，细胞密度低及稳定性差。细胞密度决定目的产物的表达量，提高悬浮细胞密度以增加表达量一直是突破口，研究人员从减少细胞凋亡、减少细胞黏连和减轻剪切力对细胞的损伤等角度提高细胞密度。其次，生产工艺的规范化和标准化，以及适用于不同细胞系的生物反应器和培养条件的优化仍在不断探索中。另外，悬浮驯化周期长，贴壁细胞驯化到稳定传代的悬浮细胞周期很长，限制了目的产物的表达，随着生物技术的不断发展，利用组学分析，寻找阻碍驯化的关键黏附因子和凋亡相关分子，以期通过基因编辑改造细胞使其快速悬浮驯化。此外，细胞在全悬浮驯化过程中的基因表达调控机制尚未被完全阐明。最后，悬浮细胞培养基的成分也需要根据细胞基因表达情况的变化进行调整优化。虽然细胞悬浮培养技术还有诸多挑战，但是随着科学技术的不断发展，相信未来能在提高细胞密度和目的蛋白表达量、缩短驯化周期和降低生产成本方面有新的突破，为细胞悬浮培养技术在重组蛋白等生物制品生产以及细胞治疗等方面的应用提供技术支持。

## 参考文献 (References)

[1] ROUX W. Beiträge zur entwickelungsmechanik des embryo [J]. Archiv Für Mikroskopische Anatomie, 1887, 29(1): 157-212.

[2] DERAKHTI S, SAFIABADI-TALI S H, AMOABEDINY G, et al. Attachment and detachment strategies in microcarrier-based cell culture technology: a comprehensive review [J]. Mater Sci Eng C

Mater Biol Appl, 2019, 103: 109782.
[3] VON DEN EICHEN N, BROMIG L, SIDARAVA V, et al. Automated multi-scale cascade of parallel stirred-tank bioreactors for fast protein expression studies [J]. J Biotechnol, 2021, 332: 103-13.
[4] RAMONET F, HADDADI B, HARASEK M. Optimal design of double stage internal loop air-lift bioreactor [J]. Energies, 2023, 16(7): 3267.
[5] BARTON R R, VANTREECK K E, DURAN C J, et al. A falling film bioreactor (FFBR) for generating effective gas-to-liquid mass transfer using wavy laminar flow for continuous microbial gas processing [J]. Chem Eng Sci, 2020, 219: 115592.
[6] KUNDU A M, HILLER G W. Hydrocyclones as cell retention devices for an N-1 perfusion bioreactor linked to a continuous-flow stirred tank production bioreactor [J]. Biotechnol Bioeng, 2021, 118(5): 1973-86.
[7] KLÖCKNER W, DIEDERICHS S, BÜCHS J. Orbitally shaken single-use bioreactors [J]. Adv Biochem Eng Biotechnol, 2014, 138: 45-60.
[8] USLU U, ERDMANN M, WIESINGER M, et al. Automated Good Manufacturing Practice-compliant generation of human monocyte-derived dendritic cells from a complete apheresis product using a hollow-fiber bioreactor system overcomes a major hurdle in the manufacture of dendritic cells for cancer vaccines [J]. Cytotherapy, 2019, 21(11): 1166-78.
[9] KWON T, YAO R, HAMEL J P, et al. Continuous removal of small nonviable suspended mammalian cells and debris from bioreactors using inertial microfluidics [J]. Lab Chip, 2018, 18(18): 2826-37.
[10] SYED M S, MARQUIS C, TAYLOR R, et al. A two-step microengineered system for high-density cell retention from bioreactors [J]. Sep Purif Technol, 2021, 254: 117610.
[11] WIEGMANN V, MARTINEZ C B, BAGANZ F. Using a parallel micro-cultivation system (micro-matrix) as a process development tool for cell culture applications [J]. Methods Mol Biol, 2020, 2095: 69-81.
[12] 代为俊, 刘旭平, 周燕. 基于MDCK悬浮细胞生产禽流感疫苗的放大工艺开发[J]. 中国兽药杂志(DAI W J, LIU X P, ZHOU Y. Development of scale up for the production of influenza vaccine based on MDCK suspension cells [J]. Chinese Journal of Veterinary Drug), 2017, 51(8): 6.
[13] 杨惠清, 武发菊, 葛玉凤, 等. 无血清全悬浮培养Vero细胞系的建立及生物反应器培养参数优化[J]. 动物医学进展(YANG H Q, WU F J, GE Y F, et al. Establishment of Vero cell line in serum-free suspension culture and optimization of bioreactor culture parameters [J]. Progress in Veterinary Medicine), 2023, 44(1): 54-9.
[14] HAN Y H, WANG Y, LEE S J, et al. Regulation of anoikis by extrinsic death receptor pathways [J]. Cell Commun Signal, 2023, 21(1): 227.
[15] DAI X, MIAO Y, HAN P, et al. PABPC1 enables cells with the suspension cultivation feature [J]. ACS Synth Biol, 2021, 10(2): 309-17.
[16] HARJUNPÄÄ H, LLORT ASENS M, GUENTHER C, et al. Cell adhesion molecules and their roles and regulation in the immune and tumor microenvironment [J]. Front Immunol, 2019, 10: 1078.
[17] MACDONALD M A, BARRY C, GROVES T, et al. Modeling apoptosis resistance in CHO cells with CRISPR-mediated knockouts of Bak1, Bax, and Bok [J]. Biotechnol Bioeng, 2022, 119(6): 1380-91.
[18] TANG D, LAM C, BAUER N, et al. Bax and Bak knockout apoptosis-resistant Chinese hamster ovary cell lines significantly improve culture viability and titer in intensified fed-batch culture process [J]. Biotechnol Prog, 2022, 38(2): e3228.
[19] PECH S, REHBERG M, JANKE R, et al. Tracking changes in adaptation to suspension growth for MDCK cells: cell growth correlates with levels of metabolites, enzymes and proteins [J]. Appl Microbiol Biotechnol, 2021, 105(5): 1861-74.
[20] SÈNE M A, XIA Y, KAMEN A A. Comparative transcriptomic analyses of a Vero cell line in suspension versus adherent culture conditions [J]. Int J Cell Biol, 2023, 2023: 9364689.
[21] CHU C, BOTTARO D P, BETENBAUGH M J, et al. Stable ectopic expression of ST6GALNAC5 induces autocrine MET activation and anchorage-independence in MDCK cells [J]. PLoS One, 2016, 11(2): e0148075.
[22] VAN WEZEL A L. Growth of cell-strains and primary cells on micro-carriers in homogeneous culture [J]. Nature, 1967, 216(5110): 64-5.
[23] HUANG L, XIAO L, JUNG POUDEL A, et al. Porous chitosan microspheres as microcarriers for 3D cell culture [J]. Carbohydr Polym, 2018, 202: 611-20.
[24] WANG Z, ZHANG X, XUE L, et al. A controllable gelatin-based microcarriers fabrication system for the whole procedures of MSCs amplification and tissue engineering [J]. Regen Biomater, 2023, 10: rbad068.
[25] LERMAN M J, LEMBONG J, MURAMOTO S, et al. The evolution of polystyrene as a cell culture material [J]. Tissue Eng Part B Rev, 2018, 24(5): 359-72.
[26] TASOGLU S, YU C H, GUNGORDU H I, et al. Guided and magnetic self-assembly of tunable magnetoceptive gels [J]. Nat Commun, 2014, 5(1): 4702.
[27] SINCLAIR A, O'KELLY M B, BAI T, et al. Self-healing zwitterionic microgels as a versatile platform for malleable cell constructs and injectable therapies [J]. Adv Mater, 2018, 30(39): e1803087.
[28] LIZANA-VASQUEZ G D, ARRIETA-VIANA L F, MENDEZ-VEGA J, et al. Synthetic thermo-responsive terpolymers as tunable scaffolds for cell culture applications [J]. Polymers, 2022, 14(20): 4379.
[29] CHEN X Y, CHEN J Y, TONG X M, et al. Recent advances in the use of microcarriers for cell cultures and their *ex vivo* and *in vivo* applications [J]. Biotechnol Lett, 2020, 42(1): 1-10.
[30] XU P, XU S, HE C, et al. Applications of small molecules in modulating productivity and product quality of recombinant proteins produced using cell cultures [J]. Biotechnol Adv, 2020, 43: 107577.
[31] WU S, RISH A J, SKOMO A, et al. Rapid serum-free/suspension adaptation: medium development using a definitive screening design for Chinese hamster ovary cells [J]. Biotechnol Prog, 2021, 37(4): e3154.
[32] JANG M, PETE E S, BRUHEIM P. The impact of serum-free culture on HEK293 cells: from the establishment of suspension and adherent serum-free adaptation cultures to the investigation of growth and metabolic profiles [J]. Front Bioeng Biotechnol, 2022, 10: 964397.
[33] OGAWA I, ONOZATO D, ANNO S, et al. Suspension culture of human induced pluripotent stem cell-derived intestinal organoids using natural polysaccharides [J]. Biomaterials, 2022, 288: 121696.
[34] YANKASKAS C, BALHOUSE B, PAUL C, et al. Abstract 4251:

establishment of hormone-dependent endometrial tumoroids in a conditioned-medium free, serum-free medium [J]. Cancer Res, 2024, 84(6_Supplement): 4251.

[35] CAPSTICK P B, TELLING R C, CHAPMAN W G, et al. Growth of a cloned strain of hamster kidney cells in suspended cultures and their susceptibility to the virus of foot-and-mouth disease [J]. Nature, 1962, 195(4847): 1163-4.

[36] ZHANG J, QIU Z, WANG S, et al. Suspended cell lines for inactivated virus vaccine production [J]. Expert Rev Vaccines, 2023, 22(1): 468-80.

[37] BISSINGER T, FRITSCH J, MIHUT A, et al. Semi-perfusion cultures of suspension MDCK cells enable high cell concentrations and efficient influenza A virus production [J]. Vaccine, 2019, 37(47): 7003-10.

[38] PARK S, KIM J Y, RYU K H, et al. Production of a foot-and-mouth disease vaccine antigen using suspension-adapted bhk-21 cells in a bioreactor [J]. Vaccines, 2021, 9(5): 505.

[39] WANG P, HUANG S, HAO C, et al. Establishment of a suspension MDBK cell line in serum-free medium for production of Bovine alphaherpesvirus-1 [J]. Vaccines, 2021, 9(9): 1006.

[40] PICHON S, MOUREAU A, PETIT C, et al. Safety and immunogenicity of a serum-free purified Vero rabies vaccine in comparison with the rabies human diploid cell vaccine (HDCV; Imovax® Rabies) administered in a simulated rabies post-exposure regimen in healthy adults [J]. Vaccine, 2024, 42(10): 2553-9.

[41] OKEMOTO-NAKAMURA Y, SOMEYA K, YAMAJI T, et al. Poliovirus-nonsusceptible Vero cell line for the World Health Organization global action plan [J]. Sci Rep, 2021, 11(1): 6746.

[42] RAMASAMY V, DUVVURI P K, KAUSHIK Y. Vero cell derived novel inactivated Japanese encephalitis vaccine JENVACR [J]. Int J Infect Dis, 2016, 45: 418.

[43] 查银河, 王芳, 何玉龙, 等. 基于生物反应器的猪流行性腹泻病毒(ZJ/15株)悬浮培养工艺研究[J]. 黑龙江畜牧兽医(ZHA Y H, WANG F, HE Y L, et al. Study on suspension culture technology of Porcine epidemic diarrhea virus (ZJ/15 strains) based on bioreactor [J]. Heilongjiang Animal Science and Veterinary), 2024(7): 11-7.

[44] NIE J, SUN Y, PENG F, et al. Production process development of pseudorabies virus vaccine by using a novel scale-down model of a fixed-bed bioreactor [J]. J Pharm Sci, 2020, 109(2): 959-65.

[45] FULBER J P C, FARNÓS O, KIESSLICH S, et al. Process development for newcastle disease virus-vectored vaccines in serum-free Vero cell suspension cultures [J]. Vaccines, 2021, 9(11): 1335.

[46] 庞兴, 马金霞, 刘淦, 等. 轮状病毒LLR株培养条件的优化[J]. 微生物学免疫学进展(PANG X, MA J X, LIU G, et al. Optimization of culture conditions of rotavirus strain LLR [J]. Progress in Microbiology and Immunology), 2021, 49(3): 5.

[47] 漆世华, 韩兴, 秦红刚, 等. ST细胞全悬浮培养的驯化及其培养伪狂犬病毒的工艺研究[J]. 中国兽药杂志(QI S H, HAN X, QIN H G, et al. Generation of ST cells to suspension culture and its process of culturing pseudorabies virus [J]. Chinese Journal of Veterinary Drug), 2021, 55(4): 6.

[48] 李厚伟, 王蕾, 张先锋, 等. 猪丁型冠状病毒在悬浮培养猪肾细胞LLC-PK1上的增殖特性分析[J]. 畜牧兽医学报(LI H W, WANG L, ZHANG X F, et al. Analysis of the proliferation characteristics of Porcine deltacoronavirus on suspension cultured Porcine kidney cell LLC-PK1 [J]. Acta Veterinaria et Zootechnica Sinica), 2022, 53(6): 5.

[49] 刘天伦. 无血清全悬浮PK15细胞培养猪圆环病毒2型的研究[J]. 中国兽药杂志(LIU T L. Study on suspended PK15 cells culturing Porcine circovirus type 2 with serum-free medium [J]. Chinese Journal of Veterinary Drug), 2022, 56(1): 1-6.

[50] 李家冬, 王弘. 重组蛋白正确折叠与修饰的提高策略[J]. 生物工程学报(LI J D, WANG H. Strategies to improve the folding and modification of recombinant proteins: a review [J]. Chinese Journal of Biotechnology), 2017, 33(4): 591-600.

[51] PULIX M, LUKASHCHUK V, SMITH D C, et al. Molecular characterization of HEK293 cells as emerging versatile cell factories [J]. Curr Opin Biotechnol, 2021, 71: 18-24.

[52] HACKER D L, ORDÓÑEZ-MORÁN P. Large-scale production of recombinant noggin and R-Spondin1 proteins required for the maintenance of stem cells in intestinal organoid cultures [J]. Methods Mol Biol, 2020, 2171: 171-84.

[53] 李强, 陈明, 黄飚, 等. 重组人可溶性TIM-3稳转细胞株的构建及其分泌蛋白的表达[J].中国细胞生物学学报(LI Q, CHEN M, HUANG B, et al. Construction of recombinant human soluble TIM-3 cell line and expression of its secretory protein [J]. Chinese Journal of Cell Biology), 2020, 42(7): 1194-200.

[54] BORSI G, MOTHERAMGARI K, DHIMAN H, et al. Single-cell RNA sequencing reveals homogeneous transcriptome patterns and low variance in a suspension CHO-K1 and an adherent HEK293FT cell line in culture conditions [J]. J Biotechnol, 2023, 364: 13-22.

[55] HUANG Z, HABIB A, ZHAO G, et al. CRISPR-Cas9 mediated stable expression of exogenous proteins in the CHO cell line through site-specific integration [J]. Int J Mol Sci, 2023, 24(23): 16767.

[56] MASUDA K, KUBOTA M, NAKAZAWA Y, et al. Establishment of a novel cell line, CHO-MK, derived from Chinese hamster ovary tissues for biologics manufacturing [J]. J Biosci Bioeng, 2024, 137(6): 471-9.

[57] ZAKRZEWSKI W, DOBRZYŃSKI M, SZYMONOWICZ M, et al. Stem cells: past, present, and future [J]. Stem Cell Res Ther, 2019, 10(1): 68.

[58] HOANG D M, PHAM P T, BACH T Q, et al. Stem cell-based therapy for human diseases [J]. Signal Transduct Target Ther, 2022, 7(1): 272.

[59] CUESTA-GOMEZ N, VERHOEFF K, DADHEECH N, et al. Suspension culture improves iPSC expansion and pluripotency phenotype [J]. Stem Cell Res Ther, 2023, 14(1): 154.

[60] ACKERMANN M, RAFIEI HASHTCHIN A, MANSTEIN F, et al. Continuous human iPSC-macrophage mass production by suspension culture in stirred tank bioreactors [J]. Nat Protoc, 2022, 17(2): 513-39.

[61] BRAAM M J S, ZHAO J, LIANG S, et al. Protocol development to further differentiate and transition stem cell-derived pancreatic progenitors from a monolayer into endocrine cells in suspension culture [J]. Sci. Rep, 2023, 13(1): 8877.

[62] SOUGAWA N, MIYAGAWA S, SAWA Y. Large-scale differentiation of human induced pluripotent stem cell-derived cardiomyocytes by stirring-type suspension culture [J]. Methods Mol Biol, 2021, 2320: 23-7.

[63] GUAN J, WANG G, WANG J, et al. Chemical reprogramming of human somatic cells to pluripotent stem cells [J]. Nature, 2022, 605(7909): 325-31.