基于微流控芯片的阿尔茨海默病模型构建和应用研究进展

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摘要: 阿尔茨海默病 (Alzheimer's disease, AD) 是一种进行性神经退行性疾病, 与大脑的思维、学习和记忆等功能障碍有关。AD病理特征多、病因复杂, 构建合适的病理模型对于AD的研究至关重要。微流控芯片技术将多种功能单元集成于芯片上, 可实现接近生理环境的微环境控制, 在AD病理模型构建、早期诊断以及药物筛选等方面具有良好的应用价值。本文基于AD的病理学特征, 从细胞类型、培养方式和芯片结构等角度重点综述了AD微流控芯片模型的构建, 以及微流控芯片在AD应用中的研究进展, 为进一步阐明AD机制和药物开发提供参考。

关键词: 微流控芯片; 阿尔茨海默病; 模型构建; 病理机制; 药物筛选

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Advances in the construction of models and applications of Alzheimer's disease based on microfluidic chips

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with dysfunctions related to thinking, learning, and memory of the brain. AD has multiple pathological characteristics with complicated causes, constructing a suitable pathological model is crucial for the research of AD. Microfluidic chip technology integrates multiple functional units on a chip, which can realize microenvironmental control similar to the physiological environment. It is well applied in the construction of pathological model, early diagnosis as well as drug screening of AD. This paper focuses on the construction of AD microfluidic chips model from the perspective of cell type, culture formats and the chips structure as well as the research progress of microfluidic chips in AD application based on the pathological characteristics of AD, which will provide a reference for further elucidation of AD mechanism and drug development.

Key words: microfluidic chip; Alzheimer's disease; model construction; pathological mechanism; drug screening

阿尔茨海默病 (Alzheimer's disease, AD) 是一种进行性神经系统退行性疾病, 正迅速地成为本世纪最昂贵、致命和负担重的疾病之一。据统计, 到2050年, 全世界的痴呆症患病率将增加两倍, 而根据AD的生物学 (非临床诊断) 定义, AD的实际患病率可能要高出3倍[1]。AD患者主要有生活功能、精神和行为的改变和认知能力下降等症状, 并逐步发展为行为、思维、理解、语言以及运动等能力上的不同程度的障碍[2,3]。其主要病理特征是β淀粉样蛋白 (amyloid-β, Aβ) 斑块的病

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理性堆积、Tau蛋白高度磷酸化 (hyperphosphorylated Tau protein, p-Tau) 和p-Tau细胞内聚集形成的神经原纤维缠结 (neurofibrillary tangles, NFT) 等[4,5]。AD具有病因复杂、发病机制不完全明确等特点[6], 目前尚无治愈的方法。基于AD模型的研究能更全面再现AD的病理、生化和行为等特征改变, 对阐明AD发病机制、开发有效的AD治疗方法至关重要。

微流控芯片将微米级的微通道和具有一定功能的单元集成化, 能在微通道内实现所需尺寸、几何形状、力和分子信号梯度, 使其更具有生理相关性, 从而为研究人员提供解决与细胞、组织和疾病相关问题的工具[7], 尤其是对细胞领域研究产生了重大影响[8]。微流控芯片技术具有样本用量少、批量处理通量高、时空动态控制细胞微环境、可实现生物样本分析检测等优势, 已广泛应用于细胞生物学、药物发现等领域[9,10]。近年来, 微流控平台已被应用于细胞分析, 例如, 操纵单细胞、自动介质灌流、提供细胞微环境和外部刺激以研究细胞响应以及建立长期细胞培养系统等[11]。基于微流控芯片技术的发展和应用特点, 许多学者利用微流控芯片技术进行AD相关的研究, 本文总结了近年来微流控芯片技术在AD病理模型构建的研究进展, 以及在AD发病机制、疾病诊断和药物研发中的应用, 为进一步阐明AD发病机制和药物研发提供技术参考和科学依据。

1 阿尔茨海默病概述

1.1 病理学特征

与正常大脑相比, AD患者脑部神经病理变化较大 (图1A)。AD的神经病理学特征主要包括Aβ斑块沉积[3]、p-Tau[3]、NFT和神经元突起中的Tau蛋白的聚集体[3,12]、突触缺陷和神经元丧失造成的大脑皮层萎缩[12]、星形胶质细胞和小胶质细胞的活化[12,13]、神经炎症以及神经损伤[13]等。此外, 影响AD发病的因素还有大脑心血管系统和淋巴系统功能病变、衰老、血脑屏障 (blood-brain barrier, BBB)、外周免疫系统以及肠道菌群等[1]。AD病理机制假说较多 (图1B), 其中比较受认可的是Aβ沉积和Tau蛋白异常磷酸化[14]。

1.2 现有AD病理模型

已见文献报道的AD病理模型主要包括动物模型和体外细胞模型, 这些模型的构建多以再现AD病理生理学特征中的一种或多种, 例如淀粉样斑块的形成、Tau蛋白聚集、NFT、小胶质细胞的神经免疫反应以及BBB损伤等。

AD动物模型主要包括Tg2576、APP23、3xTg等转基因啮齿类动物模型、以黑腹果蝇和秀丽隐杆线虫等为代表的无脊椎动物模型和以斑马鱼模型为代表的模式生物模型等[15]。AD的病程长, 神经系统病理变化多, 但没有明确的发病机制或病因。在构建AD动物模型时, 除了病理变化的累积外, 还需考虑不同病理特征的发生顺序和认知障碍等行为变化[16]。理想的AD动物模型对于发现治疗靶点、揭示AD的发病机制以及抗AD药物的研发具有重要意义[17]。当前已有以啮齿类动物为代表的多种动物模型用于阐明AD发病机制[18,19]。然而, 现有AD动物模型并不能完整模拟人类AD病理过程的诸多因素。由于基于动物模型的药物筛选方法和临床筛选方法之间存在差异, 导致在临床前阶段抗AD效果明显的候选药物在临床试验中常常失败[17,20]。

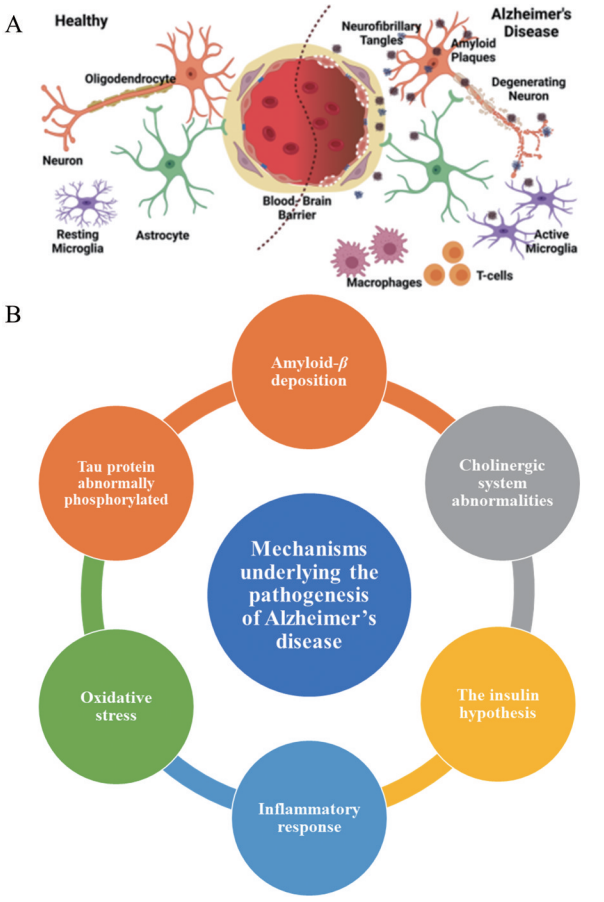

Figure 1 Schematic diagram of neuropathological characteristics and pathological mechanism hypotheses of Alzheimer's disease (AD). A: Comparison neuropathological changes occurring in the brain between healthy individuals and AD patients[13]. Reprinted with permission from reference[13]. Copyright © 2021 Wiley-VCH GmbH. B: The pathogenesis of AD

AD细胞模型是一个相对简化的平台, 能通过可控的条件观察和测试AD的病理和生物学过程, 对于AD发病机制相关蛋白的生物学功能研究具有重要意义[21]。AD的体外研究已广泛从分子和细胞水平阐明

AD发病机制及其治疗药物筛选。用于AD细胞模型构建的细胞主要有星形胶质细胞、小胶质细胞、内皮细胞和神经元细胞、诱导多能干细胞 (induced pluripotent stem cell, iPSC)、人脑微血管内皮细胞 (HBMEC、HCMEC)、神经干细胞以及神经母细胞瘤细胞 (SH-SY5Y) 等[13]。但现有简单的细胞培养系统不能模拟人脑的复杂环境以及与其他非神经细胞的相互作用[22-24]。因此, 为探究多因素对AD疾病病理和生化特征的影响, 提高AD药物研究效率, 研究者们设计和构建了更全面的体外模型进行AD相关研究。

2 微流控芯片AD病理模型的构建

目前, 在体外模拟AD等高度复杂的中枢神经系统疾病仍是巨大的挑战。传统的动物模型和体外细胞模型对研究AD都有一定的局限性[25]。利用微流控芯片技术在体外构建具备准生理环境的AD模型为AD研究提供独特的解决方案。AD微流控芯片模型主要包含芯片结构、细胞培养和诱导AD病理特征的因子等要素, 模型构建通过细胞类型、细胞3D培养和芯片结构等模拟大脑的结构和功能, 并研究脑微环境和神经血管单元之间复杂的相互作用。

2.1 细胞类型

建立AD病理模型需要了解大脑功能单元的细胞类型、内环境组成及其之间相互作用。使用与AD发病机制及病理特征相关的细胞, 如神经元细胞、星形胶质细胞、小胶质细胞、脑内皮细胞以及BBB中的细胞等[13], 加入细胞外基质或支架, 将其接种入微流控芯片系统中, 经过一段时间培养后, 加入诱导并表达AD病理特征的相关因素, 逐步形成较完整的AD微流控芯片模型。

AD几乎涉及神经元、星形胶质细胞和小胶质细胞等主要脑细胞类型的复杂相互作用[26], 这些细胞的病变和基因表达与AD进展高度相关, 因此, 这些类型的脑细胞是较理想的AD造模来源。神经干细胞是一种多能、可自我更新的细胞, 在发育过程中可以分化为神经元、星形胶质细胞和少突胶质细胞[27], 使用神经干细胞来替换和恢复受损的胆碱能神经元和大脑连接可能会为AD治疗提供新的选择[28,29]。SH-SY5Y可用于研究AD和Tau病变的相关生理学和病理学, 也可以使用各种物质分化成神经元样细胞[30], 但未分化的SH-SY5Y细胞的糖酵解表型与原代神经元细胞有所不同, 并缺乏对谷氨酸和*N*-甲基-*D*-天冬氨酸的兴奋性[31], 因此, 分化成更接近体内成熟神经元的SH-SY5Y细胞更适用于与AD相关的建模和机制研究。

由于动物细胞与人类细胞在蛋白质功能、信号通路等存在差异, 利用动物细胞构建的AD模型, 其研究结果与临床实际应用有一定差距。近年来, 人iPSC分化为包括脑细胞在内的身体各类细胞的技术出现开启了神经退行性疾病研究的新纪元[20], iPSC分化的细胞类型已经广泛地应用于AD模型及疾病研究[32]。几乎所有主要的脑细胞类型都可以由iPSC分化而来, AD患者的许多细胞功能可以在体外用iPSC分化的细胞重现, 利用共培养平台可以更深入了解神经病变过程中脑细胞之间发生的相互作用; 此外, 患者iPSC分化的神经元能代表其独特的离体神经元网络, 具有个体化治疗的潜在应用, 可以在细胞微环境中研究疾病相关的表型、生物标志物和药物筛选等[33-35]。总之, 基于iPSC分化的多种脑细胞之间的相互作用研究及共培养的优势, 能更准确地在体外使用人源的细胞构建AD病理学模型和研究疾病机制[33,36,37], 并在临床个体化治疗方案开发和药物筛选具有潜在应用[38]。Lomoio等[39]利用来自AD患者的iPSC细胞, 将丝素蛋白组成的多孔支架与插入的胶原水凝胶相结合, 构建了iPSC的3D生物工程神经组织模型, 可以长时间支持神经元和神经胶质细胞生长成复杂且具功能性的神经网络, 实验结果发现, AD模型中2个月和4.5个月时的神经元过度兴奋和$A\beta_{42/40}$比率等现象与AD患者大脑观察到的相似, 表明该iPSC模型成功模拟了时间依赖性AD相关表型。Vatine等[40]通过将器官芯片技术与人iPSC分化的脑微血管内皮细胞、星形胶质细胞和神经元结合, 构建了一个可以概括复杂的BBB功能的神经血管单元, 为BBB相关疾病建模、BBB的作用、药物筛选以及个体化医学提供研究平台。

2.2 细胞3D培养模式

大脑中多种细胞如神经元、星形胶质细胞和小胶质细胞等分布在明确的3D环境中, 存在不同细胞之间的接触及细胞与细胞外基质的相互作用, 形成一个动态和高度复杂的网络, 调节其中的细胞状态和功能[13]。

20世纪以来, 2D细胞培养是评估不同疾病系统的最常用模型之一, 其主要缺点是不能模拟神经细胞的高度组织化[41], 在体外不能准确地反映疾病条件下的细胞特征[10]。多数2D培养的AD细胞模型缺乏细胞–细胞间接触和相互作用的3D脑组织的基本特征。3D培养可以在体外模拟体内的细胞形态、细胞附着、增殖、对刺激的反应、分化、药物代谢以及细胞间质流动等特征[42-44]。3D培养的细胞外基质和生化效应能提供一定的机械强度和完整性, 维持神经区域的动态平衡, 形成高度复杂的网络, 在调节神经干细胞分化、神经元迁移以及中枢和外周突触的成熟和功能等过程中发挥关键作用[45,46]。3D细胞培养的目标是在体内和传统的体外2D培养之间架起一座桥梁, 提供与体内生理更相关的研究平台[47]。已有报道的体外3D培养的AD

模型包括神经球体系统、微流控芯片培养系统、类器官培养系统、3D水凝胶系统、3D生物打印系统以及体外神经血管单元生物工程模型等[4,48-53]。其中, 以微流控芯片技术为基础的3D培养模式可以在细胞、器官或多器官水平上模拟体内微环境, 使对细胞培养环境敏感的神经元长期培养成为可能, 为AD的神经元相互作用、功能和信号通路等研究提供更可靠的平台[54,55]。

2.3 芯片结构

2.3.1 基于3D共培养的芯片结构 共培养模式可以研究两种或两种以上细胞的相互作用。传统的2D共培养系统无法在空间上定位细胞或细胞部分 (例如, 神经元的轴突和胞体), 也无法选择性地分析和探测特定细胞[56]。利用微流控芯片技术可设计能模拟AD病理特征的3D培养和共培养条件特定区室的病理模型。

2015年, Park等[44]首次报道了3D共培养的微流控芯片AD模型。通过微流控芯片将管状脑内皮细胞 (BECs) 与3D分化的ReN-AD细胞共培养, 并将凹型微孔阵列与微渗透泵系统结合, 构建了一种生理相关的3D神经球体微流控脑芯片模型 (图2A), 分别研究了静态培养和动态培养条件下神经球体的大小和神经网络形成的变化。其中的芯片凹型微孔用于形成大小均匀的3D神经球体, 渗透泵提供持续的介质流动, 通过3D结构和介质流动模拟正常和AD大脑的微环境。结果发现, 在动态条件下培养的神经球体比在静态条件下培养的球体更大、神经网络更强健; 利用此模型还研究了Aβ对神经毒性的影响, 发现细胞存活率下降、神经损伤和突触功能障碍增加, 与体内AD的病理生理特征高度相关, 并可以在一个微流控芯片平台上同时模拟正常大脑和AD大脑。该芯片模型能模拟体内微环境的间质水平的流动, 并在不需要其他辅助设备的情况下进行长期的体外观察, 对了解AD或其他神经系统疾病的病理或治疗策略的研究有较好的应用前景。

Park等[57]利用微流控芯片技术建立了由人神经元、星形胶质细胞和小胶质细胞组成的3D培养的AD模型 (图2B), 该模型由两个模拟体内AD环境的腔室构成, 即中央室的神经元和星形胶质细胞以及角室的小胶质细胞, 并可观察细胞间的相互作用。将过表达Aβ前体蛋白的人神经前体细胞分化为神经元和星形胶质细胞, 并表达Aβ聚集、p-Tau形成等AD的病理特征, 以及伴随着CCL2、肿瘤坏死因子-α和干扰素-γ等趋化因子/细胞因子增加诱导小胶质细胞的聚集, 从而导致显著的神经元或星形胶质细胞损伤。此模型可运用于AD大脑中神经−胶质细胞相互作用基本机制和药物发现研究。McQuade等[58]利用这个3D微流控芯片AD模型比较了iPSC-小胶质细胞对AD信号的趋化作用, 测定了野生型和基因敲除的小胶质细胞向可溶性Aβ或产生Aβ的人神经元和神经胶质的混合培养物的迁移, 结果发现, 基因敲除的小胶质细胞向更具生理特性的含$A\beta_{1\text{-}40}$和$A\beta_{1\text{-}42}$的混合培养物的迁移受损更显著, 且减少了周围淀粉样斑块的聚集, 推测小胶质细胞在AD发病过程中起重要作用。

2.3.2 基于血脑屏障单元的芯片结构 BBB是内源性物质和治疗药物分子进入脑组织的主要屏障。BBB的高度选择透过性主要是由于其独特的结构, 包括排列在血管内的内皮细胞、与基底膜相关的周细胞与内皮细胞紧密连接和将其末端延伸到血管近腔侧的星形胶质细胞足突[59,60]。同时, BBB内皮细胞中外排泵的表达可以阻止小分子在细胞膜上的扩散。BBB可以保护神经组织免受血液中毒素的影响, 但也限制了治疗药物的通过, 其复杂性也阻碍了能模拟其健康状态和疾病特征的模型的开发。目前已有学者研究了BBB体内模型 (动物模型)[61,62]、2D细胞模型[63-65]和体外3D模型[66-68], 其中, 使用相关细胞系构建的体外3D培养Transwell模型和微流控芯片模型等BBB模型, 为跨BBB研究及中枢神经系统疾病药物研发提供了更具生理相关性的模型。

越来越多研究表明, BBB功能损伤是中枢神经系统认知功能障碍的主要原因之一, BBB是AD中神经病变、血管损伤和炎症之间的纽带[69], BBB芯片模型将是研究Aβ的转运及AD应用的理想平台[70]。基于BBB微流控芯片的AD模型能在体外模拟AD中BBB破坏和药物评估的研究。Shin等[71]在3D微流控芯片中通过具有BBB样表型的管状BECs屏障模拟脑血管, 与3D分化的ReN-AD细胞共培养组成具有完整血管壁的3D培养的AD模型 (图3), 研究AD病理微环境对BBB的主要组成部分BECs的直接影响。结果发现, 此模型成功模拟了AD患者的一些血管病变, 如BBB通透性增加、紧密连接蛋白、claudin-1和claudin-5、黏附连接蛋白、VE-cadherin的表达减少、基质金属蛋白酶-2和活性氧类水平增加以及Aβ聚集等。在内皮通道中加入了凝血酶, 凝血酶通过渗漏的BBB加剧AD的神经元损伤; 神经元中淀粉样蛋白前体蛋白 (amyloid precursor protein, APP) 和早老蛋白-1基因的突变导致Aβ大量分泌, 并可观察到其沉积在内皮细胞表面, 与屏障通透性的增加相对应。上述研究结果表明, 该AD微流控芯片模型更接近体内生理病理状态, 可运用于研究AD中BBB功能障碍的机制, 进而可作为一个标准化的AD药物筛选平台。

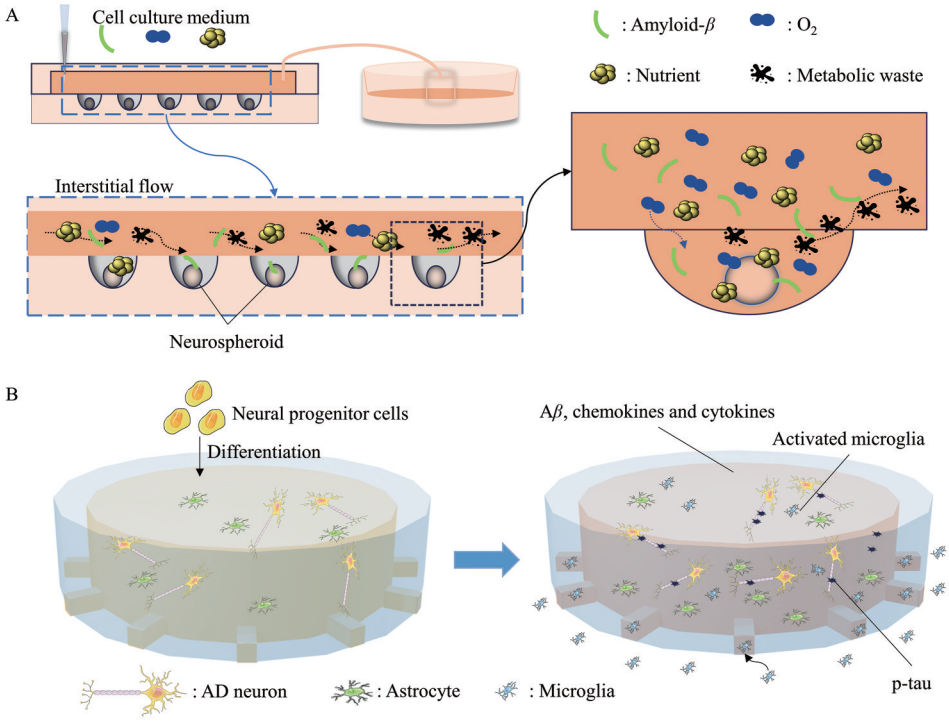

Figure 2 Schematic diagram of 3D co-culture AD microfluidic chip model. A: 3D neurospheroid AD microfluidic chip model; B: 3D human co-culture AD microfluidic model constructed of AD neurons, astrocyte and microglia, the left figure is a schematic of the 3D co-culture layout of neural progenitor cells differentiated into neurons and astrocytes and seeding in this AD microfluidic chip model, and the right figure is a schematic of the AD microfluidic chip model that can represent several AD pathological characteristics

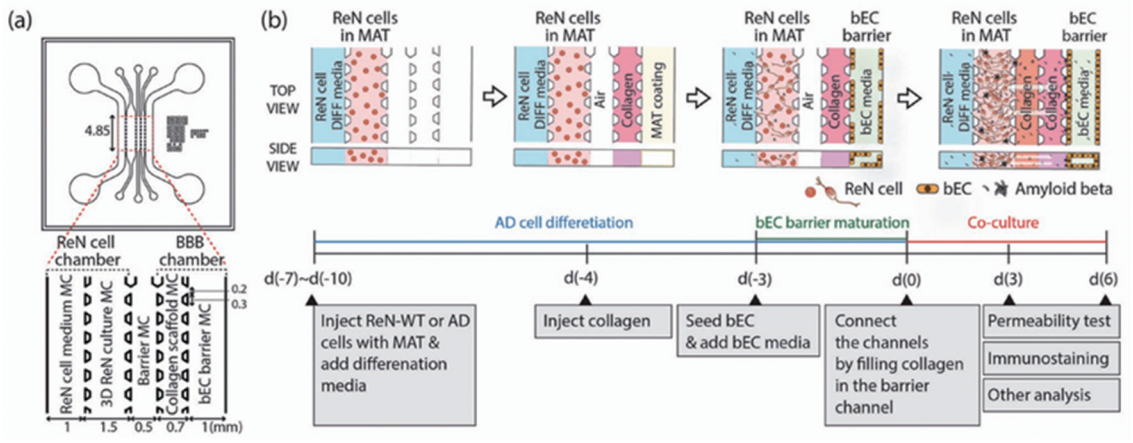

Figure 3 Schematic of the construction procedure of AD model based on BBB microfluidic chips[71]. Reprinted with permission from reference[71]. Copyright © 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

3 微流控芯片技术在AD研究中的应用

与传统的体外培养系统相比, 微流控芯片在模拟大脑多细胞、3D培养、血管化以及细胞外基质等典型的组织复杂性特征具有显著优势, 常被用来模拟和研究神经网络发育、细胞间信号传导和免疫功能等中枢神经系统的生理现象[72]; 此外, 还可设计为集成模型构建、药物疗效和潜在不良反应评价等多功能于一体的芯片平台[73]。基于上述优势, 利用微流控芯片可开发更具生理学相关性的体外模型并部分替代动物研究[74], 近年来逐渐应用于AD病理机制、早期诊断以及药物筛选等研究领域。

3.1 在AD病理机制和诊断研究中的应用

基于微流控芯片技术的优势, 根据组织和器官的自然结构和功能及特征进行设计和制作, 结合3D培养和共培养模式, 在芯片上再现与人体组织的物理结构和微环境相似的系统, 且以PDMS为代表的生物相容

性聚合物芯片材料可以实现高分辨率和实时成像。因此, 微流控芯片技术已逐渐应用于AD的生理病理学、神经毒性、疾病机制以及早期诊断等方面的研究。当前利用微流控芯片可以运用于Aβ聚集和检测、神经毒性、Aβ和小胶质细胞之间的相互作用、Tau病理、APP检测以及生物标志物发现等病理机制和诊断中的研究。Sun等[75]设计了一种金等离子体壳附着的聚苯乙烯微球的表面增强拉曼光谱免疫传感微流控芯片, 能实现同时对AD相关生物标志物A$\beta_{1\text{-}42}$和p-Tau181蛋白定量分析, 该方法检测限低、灵敏度高, 具有良好的选择性和特异性, 可在体外快速检测A$\beta_{1\text{-}42}$和p-Tau181, 辅助AD早期临床诊断。Katsikoudi等[76]使用微流控芯片研究人AD来源的Tau从神经元到神经元的传递, 构建了接种人Tau的大鼠皮质神经元微流控芯片培养模型, 利用高内涵成像定量评估该模型的Tau内含物的形成和聚集, 并测试抑制剂分子的效果, 发现小分子聚集抑制剂可以阻断Tau聚集体的跨神经元传递, 表明该系统可用于评估Tau传递机制, 为AD潜在的Tau疗法研究提供平台。表1[44,57,77-95]列举了近年来微流控芯片技术在AD的病理机制和诊断研究的应用实例。

3.2 在AD药物筛选和评价中的应用

与传统研究平台相比, 微流控芯片具有体积小、分析时间短、成本相对较低以及可实现高通量药物筛选等优势[74], 目前在AD药物筛选和评价领域的应用日益增多, 包括AD候选化合物筛选、药物的渗透性评价、药效评价以及AD药物–剂量反应等。Ahn等[96]构建了一种具有生理相关结构和功能的BBB微流控芯片模型, 可以进行给药后跨内皮电阻 (transendothelial electrical resistance, TEER) 测定、纳米颗粒采样和荧光激活细胞分选分析, 实现在芯片上对纳米颗粒在脑血管及周围区域的转运和分布定量评估。Privitera等[97]采用激光诱导荧光和HPLC相结合的芯片电泳法, 研究了β-alanyl-*L*-histidine对调节活性氧产生的能力、细胞能量代谢和氧化应激参数在激活的HMC3小胶质细胞中的变化, 结果表明β-alanyl-*L*-histidine对AD的认知障碍具有治疗潜力。表2[98-106]总结了近5年报道的微流控芯片在AD药物筛选和评价中的应用及其优势。

4 小结与展望

AD的病理特征和发病机制非常复杂, 现有的AD动物模型和细胞模型不能很好地模拟其体内真实病理状态, 表3总结了AD动物模型、细胞模型和微流控芯片模型这三类模型的优缺点。微流控芯片技术的应用优势为AD病理模型研究提供了新的选择, AD微流控芯片模型构建主要考虑细胞类型、细胞培养模式和芯片结构, 利用芯片平台模拟AD的一些病理和生理学特征, 并对其进行相关表征和评估, 评价模型用于AD研究的适用性和应用前景。目前, 微流控芯片在AD研究中已广泛应用于病理学特征、疾病机制、早期诊断、药物筛选以及药效评价等。

尽管微流控芯片技术在AD模型构建和应用研究中具有独特优势, 但还是面临着一些挑战: ① 由于微流控芯片模板结构的不同、培养条件的差异以及因应用目的差异, 导致微流控芯片设备中的流体参数控制不同, 有必要针对需求考察设置合理的参数, AD的模型构建、应用及评价标准仍相对匮乏; ② 当前AD芯片模型的构建主要以BECs、神经元、星形胶质细胞以及小胶质细胞等构成神经血管单位的细胞为主, 但研究表明免疫系统可能也与AD疾病进展之间存在一定关系[107], 需要对多细胞相互作用表征, 以验证模型内细胞/组织模拟的体内条件; ③ AD病因复杂, 病因不完全明确, 完全模拟其复杂的体内条件和全部病理特征仍具有很大挑战性, 目前的AD微流控芯片模型以再现其中的一种或多种病理特征为主。

因此, 未来的应用研究可能需要增强这些模型的生理相关性和规范评价体系, 例如通过纳入其他细胞类型如相关免疫细胞、较多因素诱导或按照与体内相似不同病理现象发生顺序设计和研究, 将有助于进一步阐明各个因素在AD中的作用及应用。尽管目前基于微流控芯片的研究系统不能完全模拟AD脑部的病理特征、阐明AD大脑中神经血管单元破坏的生理和病理基础, 但作为一个跨学科和迅速发展的领域, 基于微流控芯片的研究系统有望从根本上改变AD神经退行性研究从基础科学到药物发现转化的方式, 例如微流控芯片技术和干细胞工程学相结合开发个体化模型, 预测药物对特定患者的毒性和疗效, 提高临床试验的效率, 并有助于探究患者的特异性生物标志物, 以及基于特定患者的药代动力学的个体化给药方案[108]。随着微流控芯片技术、生物学和工程学等多学科领域的融合和发展, 微流控芯片将可用于建立更仿真的AD病理模型, 探究AD的发病机制, 并为AD药物筛选、药效评价和临床个体化治疗方案制定提供技术支撑和科学依据。

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利益冲突: 所有作者均声明没有任何利益冲突。

Table 1 Application of microfluidic chips in pathological mechanisms and diagnostic studies of AD

Research population	Application	Key finding	Reference
Neural progenitor cell	Neurotoxic effects of Aβ	Decreased cell viability, increased neural destruction and synaptic dysfunction	[44]
Neurons, astrocyte and microglia	Aβ aggregation, p-Tau accumulation and neuroinflammatory activity	Microglia recruitment, neuroinflammatory response and neuron/astrocyte damages	[57]
A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$	Molecular diagnosis of AD	A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ in cerebrospinal fluid samples were successfully detected using the developed batchwise immunoassay approach	[77]
Prenatal rat neuronal cells	Studying the neurotoxicity of Aβ	Aβ oligomeric assemblies rather than fibrils have potential neurotoxicity	[78]
Primary cortical mouse neurons	The distant effects of local Aβ stress on neuronal subcompartments and networks	Aβ peptide accumulation in the somato-dendritic compartment of cortical neurons leads to a fast anterograde propagation of degenerative signals toward endings, resulting in presynaptic collapse	[79]
Microglial cell	The distinct roles of Aβ on microglial accumulation	Soluble and insoluble Aβ have synergistic effects on microglial accumulation to sites of Aβ deposits	[80]
Human serum	Label-free detection of Aβ aggregates	Enables the real-time, sensitive detection of biomarkers in bodily fluids, and will be useful for the development of portable diagnostic devices	[81]
Chinese hamster ovary (CHO) cell lines (CHO-pcDNA4, -APP^{WT} and -APP^{LDN})	A$\beta_{1\text{-}42}$-induced synaptotoxicity	Protein tyrosine kinase 2β is selectively expressed in postsynaptic neurons and prevents A$\beta_{1\text{-}42}$-induced synaptotoxicity	[82]
Biological fluids	Quantitative AD diagnosis by A$\beta_{1\text{-}42}$	Immune-capture A$\beta_{1\text{-}42}$ molecules uniformly distributed in clinically relevant volumes of fluid −100 μL − in a range of concentrations clinically relevant for AD early diagnosis	[83]
Blood plasma	Aβ and Tau protein detection for AD early diagnosis	Detected Aβ and Tau proteins in femtomolar levels and successfully applied to detect trace amount of target proteins in blood plasma	[84]
Trem2 knockout microglia	Mechanisms underlying Trem2-dependent modulation of Tau pathology	Trem2 deletion can enhance Tau trafficking, distribution and seeding through microglial exosomes	[85]
Dissociated cortical neurons (E19)	Co-pathological states of p-Tau	Generated co-pathological states of p-Tau proteins within a connected cell culture of primary cortical neurons	[86]
Neurons	Investigate Tau misfolding and propagation across connected neurons and cytotoxicity	Seed-competent misfolded Tau species does not compromise neuronal excitability, but instead initiate discrete cellular dysfunctions	[87]
Hippocampal neurons, BV2 microglial cell	Axon–glia interactions	Preferential accumulation of microglia specifically to injured as compared to healthy axons	[88]
Rat brain endothelial cells (RBE4)	Studying the roles of BBB	The tight BBB model could be disrupted by exposure to TNF-α and in conditions of ischemia	[89]
Fibroblast and human serum	Potential biomarkers for the early detection of vascular dysfunction	PTP4A3 as a potential biomarker and therapeutic target in AD by demonstrating its critical role in the regulation of the BBB permeability	[90]
Serum	A blood biomarker for the early diagnosis of AD	Achieve ultra-sensitive protein detection at levels as low as 0.35 fg·mL^{-1}; can differentiate healthy subjects from MCI subjects and AD patients with excellent specificities by detecting the ADAM10 blood level	[91]
Human plasma	Detection of AD biomarkers in human blood plasma for use in clinical AD diagnostics	Developed an acoustofluidic multimodal sensing platform for isolating and detecting AD biomarkers containing Aβ peptides and Tau proteins	[92]
Human fibroblasts	Biomarker discovery and applications in diagnosis	Exploit the chemical direct reprogramming of patient skin fibroblasts into neurons, enables on-chip examination of disease pathological processes	[93]
Blood	MicroRNA identification as a disease-stage specific biomarker and predictor	Increases in miR-206 during AD progressing towards an AD-like phenotype, demonstrates the diagnostic and prognostic potential of blood-based miRNAs for AD	[94]
Cortical neurons	Examine APP transport and localization to the pre- and post-synaptic compartments	HTT regulates APP transport in axons but not dendrites after phosphorylation by the Ser/Thr kinase Akt	[95]

Table 2 Application of microfluidic chips in AD drug screening and evaluation

Research population	Type of microfluidic chips	Application	Advantage	Reference
Tau tubulin kinase 1 (TTBK1)	On-chip electrophoretic separation system and the quantification system	Screening of hTTBK1 inhibitors	① Direct readout of substrate conversion; ② Feasible to perform kinetic study; ③ Can be applied to develop selective hTTBK1 inhibitors.	[98]
iPSC-derived human brain microvascular endothelium interfaced with primary human brain astrocytes and pericytes	Hypoxia-enhanced human BBB on-chip	Drug screening of BBB	① Formation of a stable BBB with high, *in vivo*-like permeability restriction that lasts up to 2 weeks; ② Exhibits enhanced functionalities relative to past human BBB models.	[99]
Human astrocytes, pericytes and endothelial cells	BBB-on-a-chip	Evaluation of the permeability and cytotoxicity performance of targeted gold nanorods for theranostics of AD	① Integrating a micro-TEER measuring system; ② Allow a correct read-out and cell imaging monitoring; ③ Assess the permeability of new drugs more quickly and cheaper than *in vivo* models.	[100]
Human neural glioma C6 cells, the streptozotocin-induced Alzheimer's-like rats	Microfluidic chips for synthesizing the CS/GQD NPs	Evaluation of the efficacy of graphene quantum dots (GQD) on AD	① This microfluidic device allows reproducibly synthesize ultra-small, highly monodispersed NPs; ② GQDs as therapeutic agents were successfully encapsulated into CS NPs without altering the crystalline structure of CS, and remain structurally stable at different temperatures; ③ Ease of use, rapid mixing, and an under-controlled process of NP production with low material consumption.	[101]
Insulin monomers and amyloid fibrils	Integrated far-UV compatible measurement chambers into microfluidic chips	Can be used in the study of protein misfolding and aggregation of AD	① Measurement chambers of different heights can be integrated for a wide range of concentration measurements; ② May provide time-resolved information about the protein aggregation pathway.	[102]
Methylthioninium chloride (MTC) and Tau protein	Spiral-shaped passive micromixing microfluidic chip	Investigate Tau aggregation and dose-response of MTC on-chip	① The amount of Tau protein sample used was significantly less than the usage for conventional techniques; ② The whole protein-drug assay was realized in less than two hours; ③ Cost-effective cell-free assays.	[103]
Serum and cerebrospinal fluid	Bio-techne microfluidic Ella platform	Measurement of GFAP in blood of AD	① Highly sensitive, and easy-to-use immunoassay; ② Serum GFAP levels strongly correlated with Simoa concentrations; ③ cost-effective and the measurement time for one cartridge is only around 75 min.	[104]
Human brain microvascular endothelial cells	BBB-on-chips model	Investigated the permeability of six activity components of traditional Chinese medicine	① Simple and near-physiological conditions; ② BBB structure planarized for easy observation; ③ Simulation of fluid shear stress *in vivo*.	[105]
Rat primary brain microvascular endothelial cells (ECs), pericytes, and astrocytes	3D microfluidic BBB chip	Treated the BBB model with dexamethasone, and observed protection of the BBB	① Simple, cost-effective, and scalable; ② More physiologically relevant; ③ Suitable for screening of drug candidates that target or protect the BBB.	[106]

Table 3 Comparison of some advantages and disadvantages of different AD models

Type of AD model	Advantage	Disadvantage
Animal model	① A wider diversity of biological neurological studies; ② Animal models are important to study the function of the central nervous system; ③ Enable to dissect key disease-associated cellular and molecular processes; ④ Provide critical insights into AD pathology; ⑤ High birth rates, easy maintenance and low costs involved to their short reproductive cycles.	① Ethical concerns; ② Low predictability; ③ Brain structure and cognitive function are different from humans; ④ Significant differences at the molecular and cellular levels exist between animals and humans; ⑤ Did not fully recapitulate the pathological events involved in AD progression.
Cell model	① Conventional 2D cell model system are simple and cost-effective; ② 3D cell model systems are more appropriate to model complex functions of brain; ③ Provision of cues that influence cell structure, adhesion, proliferation, signaling and mechano-transduction.	① The information from 2D culture may be far removed from human physiology; ② 3D system lack of reproducibility; ③ Lacking of cellular tension, fluid shear stress, and compression analysis; ④ The ability to evaluate drug responses in complex diseases is limited; ⑤ Genetic and biochemical assessment of the seeded cells is difficult.
Microfluidic chips model	① Consume only a small amount of samples and materials; ② Can recapitulate physiological environments under controlled flows; ③ Remarkably higher accessibility; ④ Easy to control the experimental conditions; ⑤ Enhancement of cellular viability and growth; ⑥ High predictability of specific phenomena and treatment effects; ⑦ Flexibility of possible design and integration of multi-parameter analysis; ⑧ Enables long-term 3D culture with *in situ* tracking and real-time imaging.	① Fabrication of microfluidic device requires quite complex and time-consuming prototyping, processes, and specific equipment; ② The manufacturing procedure does not support simultaneous integration with biology; ③ Models are designed to satisfy specific experimental needs and may not be suitable for wide-scale production.

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