维持间充质干细胞干性的策略与机制

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摘要 间充质干细胞(mesenchymal stem cells, MSCs)是一种具有维持自我更新能力以及多向分化潜能的多能干细胞, 同时具有低免疫原性和高便携性的特点, 是组织工程理想种子细胞的重要来源。组织工程中需要大量种子细胞, 因此对MSCs进行体外扩增至关重要。MSCs的体外培养方式多以传统二维(two-dimensional, 2D)培养为主, 然而在这种传统的2D培养过程中MSCs往往会出现自分化现象, 导致其失去干性。MSCs的干性可以通过生物反应器、超低吸附表面、悬滴培养或支架培养等三维(three-dimensional, 3D)培养方式进行维持。该文主要从3D培养方式出发, 叙述其维持MSCs干性的作用、优势及相关机制。

关键词 间充质干细胞; 干性; 3D培养; 2D培养

Strategies and Regulatory Mechanisms for Maintaining the Stemness of Mesenchymal Stem Cells

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Abstract MSCs (mesenchymal stem cells) are pluripotent stem cells with the potential to maintain self-renewal and multi-directional differentiation, and have the characteristics of low immunogenicity and high portability. They are an important source of ideal seed cells for tissue engineering. A large number of seed cells are needed in tissue engineering, so it is very important to amplify MSCs *in vitro*. The *in vitro* culture of MSCs is mainly based on traditional 2D (two-dimensional) culture. However, in the process of traditional two-dimensional culture, MSCs often occur self-differentiation, which causes them to lose stemness. The stemness of MSCs can be maintained by 3D (three-dimensional) culture methods such as bioreactor, ultra-low adsorption surface, hanging drop culture or scaffold culture. This paper mainly starts from the 3D culture method, and describes its stemness maintenance effect, advantages and related mechanisms on MSCs.

Keywords mesenchymal stem cells; stemness; three-dimensional culture; two-dimensional culture

组织工程利用细胞、生物材料、生化信号(如生长因子)和物理信号(如机械负荷)以及它们的组合以产生组织样结构, 它的目标是开发用于维持、恢复或改善受损组织功能的替代品[1]。其中, 组织工程三要素是: 种子细胞、支架和生长因子。支架用于支撑细胞生长, 生长因子协调细胞生长, 种子细胞是生物组织最基本的结构单元, 是组织工程的核心研究内容[1]。理想的种子细胞应具备取材容易、对机

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体损伤小、体外增殖能力强、植入体内后能耐受机体免疫等特点[2]。间充质干细胞(mesenchymal stem cells, MSCs)是一种未分化的多能干细胞, 具有维持自我更新的能力以及多向分化的潜能, 且具有低免疫原性和高便携性的特点, 基于此, MSCs成为治疗多种组织退行性疾病的理想细胞, 并成为组织工程的启动细胞[3]。例如, MSCs可以用于治疗骨关节炎, 促进关节软骨再生[4]。骨髓来源的间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)可以通过促进II型胶原蛋白和蛋白质多糖等细胞外基质(extracellular matrix, ECM)成分的表达来修复退行性椎间盘[5]; 三维(three-dimensional, 3D)打印甲基丙烯酸化明胶支架培养BMSCs能够实现脂肪组织稳定再生[6]。

因组织工程需要大量的种子细胞, 而直接提取的MSCs数量有限, 因此需要对其进行大量的体外扩增。然而, MSCs在体外扩增时往往会出现自分化现象, 丧失其干性, 影响其在组织工程中的应用。考虑到MSCs的多能性(干性)是其应用于组织工程的保障, 因此如何维持以及更好地保留MSCs的干性是解决组织工程所面临的临床问题的关键。

传统的维持MSCs干性的方法是基于二维(two-dimensional, 2D)培养, 但是这种方法无法很好地模拟体内微环境, 且其干性维持的效果不佳。所以, 学者们开始基于2D培养进行改善, 发现一些特殊环境(低温环境)可能有利于维持MSCs的干性。HORCHAROENSUK等[7]发现, 基于脯氨酸(一种新型冷冻保护剂)的溶液可用于MSCs的短期低温运输, 维持其干性。尽管如此, 它仍然存在很多缺点, 如无法更好地模拟体内环境, 只适于短期干性维持等, 所以其维持MSCs干性的能力有限。此外, 越来越多的证据表明, MSCs所处的细胞微环境在确定MSCs的干性特性方面起着重要作用[8-9]。3D培养能够更好地模拟细胞微环境, 也许可以更好地维持MSCs的干性, 为此学者们开始对3D培养进行探索。

近年来学者们借助一些设备, 使MSCs形成一个3D细胞团用以维持其干性。ZHANG等[10]借助微重力生物反应器开发了一种无生物材料介导的球状体形成方法, 以维持脂肪干细胞(adipose-derived stem cells, ADSCs)的干性。随着生物材料技术的发展, 学者们逐渐开发出合适的生物材料对干细胞进行3D培养以期实现这些细胞的干性维持。LI等[11]借助含有12 nm生物活性纳米粒子(bioactive nanoparticles, BNP)的藻酸盐/明胶水凝胶生物墨水进行3D生物打印以保留MSCs的干性。

鉴于此, 本文主要从3D培养方式出发, 叙述其维持MSCs干性的作用、优势及相关机制, 这或许能够为组织工程和再生医学等领域提供指导。

1 3D培养方式维持MSCs干性的研究

研究表明, 在2D培养的条件下, 生长因子或蛋白[12-13]、缺氧[14]、自噬[15]以及基因转导[16]等均可以起到一定的维持MSCs干性的作用, 然而这些方法存在不易保存、成本高、安全隐患等不足之处。并且2D培养相比于3D培养, 无法模拟真实细胞生长环境, 干性维持效果相对于3D培养有一定差距[17-20]。所以学者们开始构建3D培养环境来实现对MSCs的干性维持。3D细胞培养方式主要分为无支架材料的细胞聚集体培养和基于支架材料的细胞培养[21]。细胞聚集体的形成有多种方式, 如生物反应器培养[22]、超低吸附表面培养[23]、悬滴培养[24]等; 基于支架材料的细胞培养借助于生物材料来实现[25]。

1.1 生物反应器培养

生物反应器借助外部力量, 使其内的细胞成团, 进而形成细胞簇, 或者用来改善3D支架中的细胞生长[26]。生物反应器按外部力量种类可分为微重力生物反应器[27]、旋转式生物反应器[26]、灌注式生物反应器[28]、搅拌式生物反应器[29]和挤压式生物反应器[30]等。搅拌式生物反应器是借助于直接机械搅拌产生的剪切力使细胞成球, 然而细胞对剪切力较敏感, 因此搅拌式生物反应器易对细胞造成影响[31]。为此, 在搅拌式生物反应器的基础上衍生出了其他几种生物反应器。旋转生物反应器是通过滑轮驱动反应容器旋转达到细胞成球的目的, 减轻了对细胞的损伤。灌注式生物反应器的优势在于可以减轻静态培养下营养物质、氧气、废物交换的扩散限制[32]。微重力生物反应器可以实现大规模和高密度的细胞培养, 减少剪切应力, 并增加细胞活力[33]。研究发现, 微重力生物反应器培养ADSCs球体可以增强其干性[10]。

1.2 超低吸附表面培养

超低吸附系统通常是使用涂有非细胞吸附生物材料的细胞培养皿来抑制细胞或球体的附着, 促进细胞–细胞间相互作用而不是细胞–底物间相互作

用, 从而有效诱导细胞自组装形成球体[34-37]。常见的低吸附力的涂层材料有琼脂糖[38]、*N*-己酰基乙二醇壳聚糖[35]等。WU等[39]构建了比例为10:1的*N*-羟基丁二酰亚胺酯(*N*-hydroxysuccinimide, NHS)和马来酰亚胺基序的多组分共聚物涂层, 该涂层可以减少成纤维细胞生长因子2(fibroblast growth factor 2, FGF2)的结合位点(用于与马来酰亚胺反应)和更多的壳聚糖位点(用于与NHS酯反应), 有助于保持猪源ADSCs的原始干性, 但它存在球体形成效率低、难以回收以及培养时间长的局限性[40-41]。

1.3 悬滴培养

悬滴培养是一种在悬浮的培养基液滴中培养细胞, 使它们能够在液滴底部聚集并形成3D球体的技术。由于悬滴培养能够使细胞形成单个细胞球体并具有高重现性, 因此该技术已被广泛采用[42-43]。研究表明, 悬滴培养的MSCs表达了更高水平的干性相关基因(*Oct-4*、*Sox-2*和*Nanog*)[44]。但悬滴培养的培养工具又限制了它的应用, 主要体现在: 细胞培养体积小, 使得在不干扰细胞的情况下进行培养基交换变得很困难; 无法控制细胞数量/球体大小; 需使用特殊的培养板, 成本更高[45-46]。

1.4 磁悬浮培养

在磁悬浮培养系统中, 与磁性纳米粒子(magnetic nanoparticle, MNP)混合的细胞受到外部磁力作用后开始在一定的悬浮高度处积累, 当细胞聚集在相同的悬浮高度时, 细胞间相互作用增强, 导致细胞聚集从而形成球体[47-48]。这种情况会引起细胞团的几何变化并促进细胞之间的接触, 从而导致细胞在通过负磁泳形成球体的过程中在气/液界面聚集[49]。磁悬浮培养牙髓来源的间充质干细胞(dental pulp-derived mesenchymal stem cells, DPSCs)可以维持其干性[50]。磁悬浮培养方式为细胞建立了良好的培养系统, 不会影响细胞的增殖、代谢等[51-56], 但它存在无法更换细胞培养基、需要筛选细胞结构的缺点[48], 这些缺点限制了磁悬浮技术在干细胞培养中的应用。

1.5 支架培养

支架材料可以模拟一种适合细胞–细胞和细胞–基质相互作用的微环境, 对细胞黏附[57]、形态发生[58]、信号转导[59]和细胞存活[60]等多方位进行调控。支架材料的选材广泛, 如无机非金属支架、脱细胞外基质、静电纺丝纤维、水凝胶等, 这些材料都已被证明能够用于维持MSCs干性[17]。

1.5.1 无机非金属支架

无机非金属是由某些元素的氧化物、碳化物等及硅酸盐、磷酸盐等物质组成的材料, 已被证明可以维持MSCs的干性。LALWANI等[61]采用一种新颖的由自由基引发的热交联方法制造了具有高孔隙率(80%~85%)以及大孔孔径(20 nm~300 μm)的3D单壁和多壁碳纳米管支架, 研究其作为人工基质长期维持人类MSCs干性和促进人类MSCs扩增的能力, 结果表明在支架上长期扩增的ADSCs保留其干性表型, 显示出强大的多向分化能力, 即3D单壁和多壁碳纳米管支架可用于体外扩增MSCs以及维持MSCs干性。然而无机非金属支架因其机械性能差、脆性大的问题无法满足其作为理想植入物的条件[62]。

1.5.2 脱细胞外基质

脱细胞外基质(decellularized ECM, dECM)指脱去组织细胞的材料, 大多由胶原、蛋白多糖、糖蛋白和糖胺聚糖等组成, 具有一定孔隙的3D结构, 更接近原ECM结构, 具有广泛的应用前景[63]。LAI等[64]采用标准程序制备脱去骨髓基质细胞且保留3D结构的ECM, 并在其上培养人源MSCs, 结果表明该dECM能够促进MSCs自我更新状态的维持和多能性的保留。LI等[65]对脱细胞过程进行了优化, 开发了一种温和且有效的脱细胞方法, 最大限度地去除了细胞和细胞成分, 同时保留了整合的ECM, 他们发现在小鼠胚胎成骨细胞前体(mouse embryo osteoblast precursor cells, MC3T3-E1)来源的dECM上培养的BMSCs具有高的干性相关基因表达。然而, 脱细胞外基质材料存在制备过程复杂且损耗率高的问题[66]。

1.5.3 静电纺丝纤维

静电纺丝纤维支架具有高表面积比、高孔隙率、低成本以及良好的生物相容性等特点而被广泛应用。JHALA等[67]使用静电纺丝技术制备聚己内酯–壳聚糖(polycaprolactone-chitosan, PCL-CHT)纳米纤维3D支架并将其用于培养MSCs, 其中, 支架为MSCs提供了仿生微环境, 该仿生微环境有助于增加细胞间相互作用, 可促使MSCs形成球体, 使MSCs具有良好的干性。基于豆瓣提取物(watercress extract, WE)的聚己内酯–聚乙二醇(polycaprolactone-olyethylene glycol, PCL-PEG)电纺支架也被证明可以促进ADSCs的增殖, 维持其干性[68]。然而, 静电纺丝技术存在有机溶剂价格贵、不易回收等不足[69]。

1.5.4 水凝胶

水凝胶材料(藻酸盐、明胶、胶原和透明质酸等)通常能表现出良好的生物相容性, 对

氧气、营养物质等具有高渗透性[70-72], 且能够模拟天然ECM的网络结构, 被广泛用于生物医学和组织工程中[73]。在纤维蛋白水凝胶上体外培养ADSCs可以维持其多能性[25]。CHIEN等[74]利用细胞抗性聚(羧基甜菜碱)水凝胶可以与细胞黏附性精氨酸–甘氨酸–天冬氨酸(Arginine-Glycine-Aspartate, RGD)肽缀合以控制细胞对底物的亲和力的原理, 在不同浓度(5 μmol/L、5 mmol/L)的RGD修饰的聚(羧基甜菜碱)水凝胶上培养人BMSCs, 结果发现RGD浓度为5 μmol/L时, 细胞会形成3D球体, 具有更好的干细胞表型, 干性维持效果更佳。

相比于支架表面培养细胞的方法, 支架内养细胞更能模拟复杂的体内环境, 以及细胞–细胞、细胞–基质之间的相互作用。研究表明, 理想的硅离子浓度(2.59 μg/mL)更有可能维持MSCs的干性, LI等[11]将BMSCs装载到掺杂有BNP的藻酸盐/明胶生物墨水中, 利用3D打印技术制备了藻酸盐/明胶支架(图1), 通过生物材料的生物降解来连续调节干细胞干性, 结果显示BMSCs在该支架上表现出较高的干性基因表达水平。包封人源ADSCs的纤维素纳米纤维–透明质酸微胶囊也可促进细胞的干性维持(图2)[75]。

此外, 支架的物理和化学特性例如力学条件(机械强度、硬度等)、表面形貌、活性离子等, 对MSCs干性也有调控作用。LIN等[76]通过调节海藻酸盐的分子量和Ca^{2+}的浓度开发了一种具有可调应力松弛率的海藻酸钠水凝胶系统, 研究发现, 相比快速应力松弛(半衰期约为50 s)和中等应力松弛(半衰期约为500 s), 缓慢的应力松弛(应力松弛半衰期约为1 800 s)更有利于维持MSCs的干性。REN等[77]通过改变透明质酸的分子量来制备具有不同机械强度的水凝胶支架, 结果发现机械强度在200 Pa左右的水凝胶系统有利于维持封装的BMSCs的干性。相比于硬质基底, 较软的葡聚糖水凝胶可以更好地促进ADSCs的干性标志物表达[78]。支架表面的微纳米结构也可以调节MSCs的干性。PANDOLFI等[79]构建了具有纳米结构的电纺明胶贴片, 该结构能够有效维持MSCs的干性。除此之外, 一定浓度范围的活性元素也被证明能够有效调控MSCs的干性。掺杂有1%硅酸钙和1%硅酸锶生物陶瓷的海藻酸盐复合水凝胶通过释放硅离子(2.59 μg/mL)和锶离子(20.20 μg/mL)协同促进BMSCs增殖和干性维持[80]。

总之, 按照培养方式划分, 维持MSCs干性的方法主要有2D培养和3D培养方式(表1)。2D培养方式包括: 添加生长因子或蛋白、缺氧条件培养、自噬、

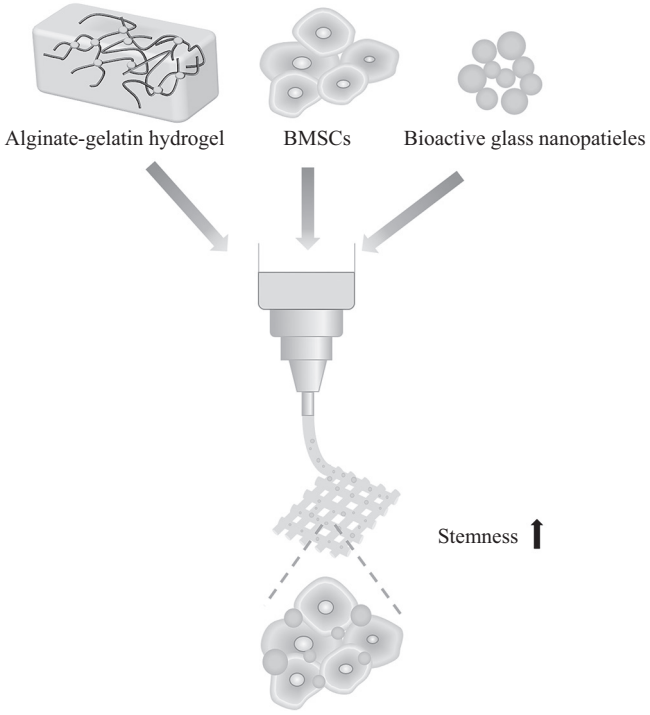

图1 3D打印装载BMSCs的含BNP的藻酸盐/明胶支架(根据参考文献[11]修改)

Fig.1 3D printing of alginate/gelatin scaffolds containing BNP loaded with BMSCs (modified from the reference [11])

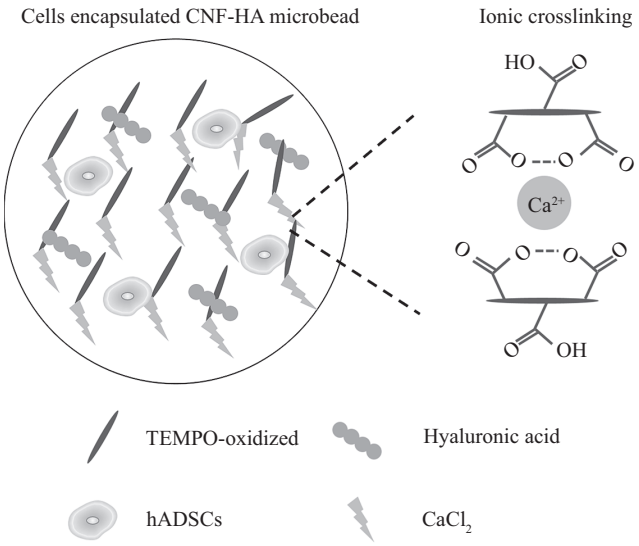

图2 装载人源ADSCs的纤维素纳米纤维–透明质酸微胶囊(根据参考文献[75]修改)

Fig.2 Cellulose nanofibers-hyaluronic acid microcapsules loaded with human ADSCs (modified from the reference [75])

表1 2D、3D培养方式维持MSCs干性

Table 1 2D and 3D culture methods to maintain the stemness of MSCs

培养方式 Cultivation methods	干性维持方法 Stemness maintenance methods	不足 Deficiencies	参考文献 References
2D culture	Growth factor	High cost, dose-dependent, security risks	[17,81]
	Protein or small molecule/drug	High cost, dose-dependent, security risks	[13,17]
	Conditioned culture (hypoxia/ serum-free culture)	Security risks, poor repeatability, many variables	[17-18,82]
	Autophagy	Security risks	[17,19]
	Gene transduction	Complex process , short gene expression time	[20,83]
	2D biomaterials	Difficult to simulate *in vivo* microenvironment	[84-85]
3D culture	Bioreactor culture	Easy to damage cells	[10,31]
	Ultra-low adsorption surface culture	Low spheroid formation rate, difficult to recycle, long incubation time	[40-41]
	Hanging drop culture	High cost, limited sphere size, difficult to mass produce	[45-46]
	Magnetic levitation culture	Cell culture volume is small and medium cannot be changed	[48,50]
	Scaffold culture		[11,17,25]

基因转导、2D生物材料等; 3D培养方式主要包括: 无支架培养方式, 该方法依赖于细胞的聚集; 有支架培养方式, 该方法依赖于支架的物理、化学等属性。这两类方式的培养过程都可以使MSCs维持优良的干性。

2 2D、3D培养方式对MSCs干性维持的比较

相比于2D培养, 3D培养可以很好地模拟体内复杂的微环境, 如结构、生理、活组织的生物信号、细胞–细胞相互作用和细胞–基质相互作用等[21,86-91]。研究表明, 3D培养在维持MSCs干性上具有很大优势。

JHALA等[67]的研究表明, 在PCL-CHT聚合物纳米纤维支架上培养的MSCs呈球状, 其在培养第5天时出现*CD44*和*CD105*标记的阳性表达, 培养10天后依然出现了这两种基因的高表达, 而2D培养的细胞没有出现这两种基因的表达, 表明与2D培养相比, 3D培养能够较长时间维持MSCs干性。与2D培养下的BMSCs相比, 3D胶原蛋白支架上培养的MSCs表现出较高的干性相关基因(*Oct4*、*Sox-2*、*Rex-1*、*Nanog*)表达, 且产生高频率的集落形成单位–成纤维

细胞(colony-forming units-fibroblastic, CFU-F), 并在诱导后显示出增强的成骨和成脂分化效率, 保持了干细胞特性[92]。与2D培养相比, 3D培养脐带间充质干细胞衍生球体在体外上调了经典多能干细胞标志物*Nanog*、*Sox-2*和*Oct-3/4*的表达水平, 高表达了*CD44*, 促进了脐带间充质干细胞干性的维持[93]。在胶原支架3D培养条件下, 绝大多数MSCs停留在细胞周期的G_0/G_1期, 而一小部分MSCs处于S和G_2/M阶段。培养几天后, 3D培养的MSCs细胞在S期的百分比显著高于2D培养的细胞[92], 而S期细胞的高百分比表明细胞在3D胶原支架上培养时增殖更为活跃[94], 从而能够说明: 相比于2D培养, 3D培养的MSCs保留了更优良的干性。

3 3D培养方式维持MSCs干性的相关机制

当用3D无支架方式培养MSCs时, MSCs形成团聚体, 球体内部的氧气浓度较低, 从而使MSCs处于缺氧状态。而当氧气浓度有限时, 细胞能够通过糖异生反应产生大量的葡萄糖, 将代谢途径从有氧转换为厌氧, 从而产生能量, 该厌氧途径称为糖酵解途径[95]。处于缺氧状态的细胞激活缺氧诱导因子-1α(hypoxia-inducible factor-1α, HIF-1α)表达, 进而激活编码葡萄糖6磷酸酶转运蛋白、乳酸脱氢酶A、糖酵解酶和葡萄糖转运蛋白的基因的表达, 以促进糖酵解途径, 实现3D无支架培养方式对干细胞干性的维持[95-96]。此外, HIF-1α还激活编码生长因子如碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)的基因, bFGF已被证明可以维持MSCs干性[97]。微重力生物反应器通过上调E-钙黏蛋白, 进而维持ADSCs的干性[10]。磁悬浮培养DPSCs可以通过激活MAPK和NF-κB信号通路, 进而维持DPSCs的干性[50]。

研究表明, 生物材料的使用可以通过传递一些物理信号(如力学信号、外部刺激等)和化学信号(如生物活性信号、小分子等), 对细胞行为和MSCs干性起到重要作用[17]。不同表型和相关微环境之间的转变会影响细胞黏附、形态和机械转导, 从而导致细胞表观遗传改变[98]。例如, 载有血小板源性生长因子-BB(platelet-derived growth factor-BB, PDGF-BB)的杜仲–明胶微球通过增加磷酸化PDGFR、磷酸化AKT和磷酸化Erk1/2的水平来促进PDGF-BB的信号转导进而维持MSCs的干性[99]。具有缓慢应力松弛的海藻酸盐水凝胶基质通过抑制FAK-PI3K/Akt-CDK1途径, 诱导MSCs进入静止状态, 从而维持其干性[76]。较低机械强度(200 Pa)的透明质酸水凝胶通过激活经典Wnt信号通路β-catenin来维持BMSCs的干性[77]。3D自组装肽水凝胶(RADA16-I)支架封装的人羊膜MSCs的干性维持可能与功能化细胞黏附配体(RGDSP、TTSWSQ和GFOGER)和不同整合素(α2、α5、α6、αv、β1和β5亚基)受体的结合, 以及整合素介导的ECM和细胞内肌动蛋白纤维之间的物理联系有关[100]。动态细胞适应性神经源性(cell-adaptable neurogenic, CaNeu)水凝胶(900 Pa)通过Yes相关蛋白(Yes-associated protein, YAP)依赖性信号转导维持封装的ADSCs的干性[101]。SWANSON等[102]的研究证明, 孔径足够小(<125 μm)的纳米纤维支架由于具有较高的主曲率有助于细胞聚集并在支架大孔内形成潜在的保护性生态位, 该支架有利于胞内YAP相关蛋白磷酸化(失活), 抑制YAP/TAZ核转位(机械敏感信号的关键介质, 依赖于YAP的核转位来激活), 有助于维持BMSCs干性。水凝胶硬度的增加会使YAP/TAZ核易位增加[103]。而底物的不同弹性对DNA甲基化模式的影响不大[104]。因此, 弹性诱导的谱系定型依赖于细胞骨架机械转导, 导致YAP/TAZ信号通路的可遗传变化, 维持MSCs干性[105]。总之, 3D培养方式维持MSCs干性的相关机制可以总结如表2。

4 总结与展望

组织工程是实现组织修复与再生的重要手段, 为临床疾病的治疗提供了新的方法和思路, 是“一场深远的医学革命”, 其中种子细胞是发挥主要功能的核心要素。然而, 间充质干细胞作为种子细胞用于组织工程仍存在着挑战: 自我更新能力不足, 在体外大量扩增时其干性容易缺失导致失去向各种组织分化的能力。因此, 学者们试图寻找和开发维持MSCs干性的新技术。在开始时, 他们通过在2D培养系统中添加生长因子、药物或蛋白, 条件培养(无血清或缺氧培养), 激活自噬以及2D材料等技术手段维持MSCs的干性。随着对MSCs干性的研究的深入, 他们开始利用3D培养系统进一步调控MSCs的干性维持。相比于2D培养, 具有3D培养环境的生物反应器培养、超低吸附表面培养、悬滴培养、磁悬浮培养以及支架培养等能够使MSCs保留更好的干性, 对组

表2 3D培养方式维持间充质干细胞干性的相关机制

Table 2 Mechanisms related to maintaining the stemness of mesenchymal stem cells by 3D culture

3D培养 3D culture	相关机制 Related mechanisms	参考文献 References
Scaffold-free culture	Activating hypoxia-inducible factor-1α	[95-97]
	Up-regulated E-cadherin	[10]
	Activating MAPK and NF-κB signaling pathway	[50]
Scaffold culture	Increasing the levels of phosphorylated PDGFR, phosphorylated AKT and phosphorylated Erk1/2	[99]
	Inhibition of FAK-PI3K/Akt-CDK1 pathway	[76]
	Activating the expression of β-catenin in the canonical Wnt signaling pathway	[77]
	Increasing expression of α2, α5, α6, αv, β1 and β5 subunit mRNAs	[100]
	Yes associated protein (YAP)-dependent signal transduction	[101]
	Increasing YAP-related protein phosphorylation and inhibit YAP/TAZ signaling pathway	[102-103]

织工程的发展和临床应用具有重要的研究意义。

虽然学者们已对MSCs干性的调控机制进行了探究, 但未来仍有一些问题需要考虑: ①同一种培养方法对不同来源的种子细胞是否具有相同的干性调控效果尚不清楚; ②单一的培养技术可能对MSCs干性长期的调控作用有限, 两种或多种培养技术相结合是否可以得到一个长期的干性维持效果还有待探索。③研究发现, MSCs的干性维持具有生长因子/蛋白/药物浓度依赖性, 但是目前对这些浓度范围尚不明确, 应该进一步探究。④生物材料在维持MSCs干性调控中已经显示出明显的优势。材料的形貌和尺寸也被证明可以调节干细胞的自我更新能力和干性维持, 但不同基质的这些参数对干细胞命运的影响可能不一致, 因此今后对这些基质参数的比较和优化值得进行深入探讨与研究。总之, 可以借助不同的方法对MSCs的干性进行维持, 以使其在组织工程中占有巨大的优势。

参考文献 (References)

[1] MHANNA R, HASAN A. Introduction to tissue engineering [J]. Tissue Eng Artif Organs, 2017, 1: 1-34.

[2] GAO G, FAN C, LI W, et al. Mesenchymal stem cells: ideal seeds for treating diseases [J]. Hum Cell, 2021, 34(6): 1585-600.

[3] 吴清法, 王立生, 吴祖泽. 间充质干细胞的来源及临床应用[J]. 军事医学科学院院刊(WU Q F, WANG L S, WU Z Z. The sources of mesenchymal stem cells and their clinical applicayions:a review [J]. Bull Acad Mil Med Sci), 2002, 26(3): 213-7.

[4] DING N, LI E M, OUYANG X B, et al. The therapeutic potential of bone marrow mesenchymal stem cells for articular cartilage regeneration in osteoarthritis [J]. Curr Stem Cell Res Ther, 2021, 16(7): 840-7.

[5] ZHANG X B, CHEN X Y, QI J, et al. New hope for intervertebral disc degeneration: bone marrow mesenchymal stem cells and exosomes derived from bone marrow mesenchymal stem cell transplantation [J]. Curr Gene Ther, 2022, 22(4): 291-302.

[6] TYTGAT L, KOLLERT M R, VAN DAMME L, et al. Evaluation of 3D printed gelatin-based scaffolds with varying pore size for MSC-based adipose tissue engineering [J]. Macromol Biosci, 2020, 20(4): 1900364.

[7] HORCHAROENSUK P, YANG-EN S, NARKWICHEAN A, et al. Proline-based solution maintains cell viability and stemness of canine adipose-derived mesenchymal stem cells after hypothermic storage [J]. PLoS One, 2022, 17(3): e0264773.

[8] LU D, LUO C, ZHANG C, et al. Differential regulation of morphology and stemness of mouse embryonic stem cells by substrate stiffness and topography [J]. Biomaterials, 2014, 35(13): 3945-55.

[9] YAN X Z, VAN DEN BEUCKEN J J, BOTH S K, et al. Biomaterial strategies for stem cell maintenance during *in vitro* expansion [J]. Tissue Eng Part B Rev, 2014, 20(4): 340-54.

[10] ZHANG S, LIU P, CHEN L, et al. The effects of spheroid formation of adipose-derived stem cells in a microgravity bioreactor on stemness properties and therapeutic potential [J]. Biomaterials, 2015, 41: 15-25.

[11] LI J, ZHANG Y, ENHE J, et al. Bioactive nanoparticle reinforced alginate/gelatin bioink for the maintenance of stem cell stemness [J]. Mater Sci Eng C Mater Biol Appl, 2021, 126: 112193.

[12] PRICOLA K L, KUHN N Z, HALEEM-SMITH H, et al. Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism [J]. J Cell Biochem, 2009, 108(3): 577-88.

[13] VICENTE LOPEZ M A, VAZQUEZ GARCIA M N, ENTRENA A, et al. Low doses of bone morphogenetic protein 4 increase the survival of human adipose-derived stem cells maintaining their stemness and multipotency [J]. Stem Cells Dev, 2011, 20(6): 1011-9.

[14] FOTIA C, MASSA A, BORIANI F, et al. Hypoxia enhances proliferation and stemness of human adipose-derived mesenchymal stem cells [J]. Cytotechnology, 2015, 67(6): 1073-84.

[15] SHI L, LI B, ZHANG B, et al. Mouse embryonic palatal mesenchymal cells maintain stemness through the PTEN-Akt-mTOR autophagic pathway [J]. Stem Cell Res Ther, 2019, 10(1): 217.

[16] NAN, DOU X, QI Y, et al. *In vitro* study of adipose-derived mesenchymal stem cells transduced with lentiviral vector carrying the brain-derived neurotrophic factor gene [J]. Int J Stem Cells, 2020, 13(3): 386-93.

[17] ZHAO C, LIN K, WANG X. Maintenance and modulation of stem cells stemness based on biomaterial designing via chemical and physical signals [J]. Appl Mater Today, 2020, 19: 100614.

[18] WERLE S B, CHAGASTELLES P, PRANKE P, et al. The effects of hypoxia on *in vitro* culture of dental-derived stem cells [J]. Arch Oral Biol, 2016, 68: 13-20.

[19] LEI Y, ZHANG D, YU J, et al. Targeting autophagy in cancer stem cells as an anticancer therapy [J]. Cancer Lett, 2017, 393: 33-9.

[20] NAKAI H, YANT S R, STORM T A, et al. Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction *in vivo* [J]. J Virol, 2001, 75(15): 6969-76.

[21] HABANJAR O, DIAB-ASSAF M, CALDEFIE-CHEZET F, et al. 3D cell culture systems: tumor application, advantages, and disadvantages [J]. Int J Mol Sci, 2021, 22(22): 12200.

[22] EGGER D, SCHWEDHELM I, HANSMANN J, et al. Hypoxic three-dimensional scaffold-free aggregate cultivation of mesenchymal stem cells in a stirred tank reactor [J]. Bioengineering, 2017, 4(2): 47.

[23] MOLYNEAUX K, WNEK M D, CRAIG S E L, et al. Physically-cross-linked poly(vinyl alcohol) cell culture plate coatings facilitate preservation of cell-cell interactions, spheroid formation, and stemness [J]. J Biomed Mater Res B Appl Biomater, 2021, 109(11): 1744-53.

[24] SON Y B, BHARTI D, KIM S B, et al. Comparison of pluripotency, differentiation, and mitochondrial metabolism capacity in three-dimensional spheroid formation of dental pulp-derived mesenchymal stem cells [J]. Biomed Res Int, 2021, 2021: 5540877.

[25] MOUSAVIFARD A, POURAZIZI NAJAFABADI E, AKHAVAN RAHNAMA M, et al. Elevated expression of stemness genes in adipose-derived mesenchymal stem cells cultured on fibrin scaffold [J]. J. Biosci, 2020, 45(1): 1-8.

[26] VARLEY M C, MARKAKI A E, BROOKS R A. Effect of rotation on scaffold motion and cell growth in rotating bioreactors [J]. Tissue Eng Part A, 2017, 23(11/12): 522-34.

[27] KOÇ A, EMIN N, ELÇIN A E, et al. *In vitro* osteogenic differentiation of rat mesenchymal stem cells in a microgravity bioreactor [J]. J Bioact Compat Polym, 2008, 23(3): 244-61.

[28] THEODORIDIS K, AGGELIDOU E, MANTHOU M, et al. Assessment of cartilage regeneration on 3D collagen-polycaprolactone scaffolds: evaluation of growth media in static and in perfusion bioreactor dynamic culture [J]. Colloids Surf B Biointerfaces, 2019, 183: 110403.

[29] NORONHA N C, MIZUKAMI A, ORELLANA M D, et al. Hypoxia priming improves *in vitro* angiogenic properties of umbilical cord derived-mesenchymal stromal cells expanded in stirred-tank bioreactor [J]. Biochem Eng J, 2021, 168: 107949.

[30] SILVA J C, MOURA C S, BORRECHO G, et al. Extruded bioreactor perfusion culture supports the chondrogenic differentiation of human mesenchymal stem/stromal cells in 3D porous poly(varepsilon-caprolactone) scaffolds [J]. Biotechnol J, 2020, 15(2): e1900078.

[31] MICHAELS J D, KUNAS K T, PAPOUTSAKIS E T. Fluid-mechanical damage of freely-suspended animal cells in agitated bioreactors: effects of dextran, derivatized celluloses and polyvinyl alcohol [J]. Chem Eng Commun, 2007, 118(1): 341-60.

[32] SCHMID J, SCHWARZ S, MEIER-STAUDE R, et al. A perfusion bioreactor system for cell seeding and oxygen-controlled cultivation of three-dimensional cell cultures [J]. Tissue Eng Part C Methods, 2018, 24(10): 585-95.

[33] SALEH F A, FRITH J E, LEE J A, et al. Three-dimensional *in vitro* culture techniques for mesenchymal stem cells [J]. Methods Mol Biol, 2012, 916: 31-45.

[34] NITTA S, HISASUE M, HORIGUCHI Y, et al. Three-dimensional spheroid culture of canine hepatocyte-like cells derived from bone marrow mesenchymal stem cells [J]. Regen Ther, 2020, 15: 210-5.

[35] JANG B S, PARK K H, SUH E Y, et al. Non-cell adhesive hexanoyl glycol chitosan hydrogels for stable and efficient formation of 3D cell spheroids with tunable size and density [J]. Int J Biol Macromol, 2021, 187: 955-63.

[36] KAMATAR A, GUNAY G, ACAR H. Natural and synthetic biomaterials for engineering multicellular tumor spheroids [J]. Polymers, 2020, 12(11): 2506.

[37] SU G, ZHAO Y, WEI J, et al. The effect of forced growth of cells into 3D spheres using low attachment surfaces on the acquisition of stemness properties [J]. Biomaterials, 2013, 34(13): 3215-22.

[38] LI M, FU T, YANG S, et al. Agarose-based spheroid culture enhanced stemness and promoted odontogenic differentiation potential of human dental follicle cells *in vitro* [J]. In Vitro Cell Dev Biol Anim, 2021, 57(6): 620-30.

[39] WU C Y, LIU H Y, HUANG C W, et al. Synergistically controlled stemness and multilineage differentiation capacity of stem cells on multifunctional biointerfaces [J]. Adv Mater Interfaces, 2017, 4(11): 1700243.

[40] LIU B H, YEH H Y, LIN Y C, et al. Spheroid formation and enhanced cardiomyogenic potential of adipose-derived stem cells grown on chitosan [J]. Biores Open Access, 2013, 2(1): 28-39.

[41] YEH H Y, LIU B H, HSU S H. The calcium-dependent regulation of spheroid formation and cardiomyogenic differentiation for MSCs on chitosan membranes [J]. Biomaterials, 2012, 33(35): 8943-54.

[42] FENNEMA E, RIVRON N, ROUWKEMA J, et al. Spheroid culture as a tool for creating 3D complex tissues [J]. Trends Biotechnol, 2013, 31(2): 108-15.

[43] KATT M E, PLACONE A L, WONG A D, et al. *In vitro* tumor models: advantages, disadvantages, variables, and selecting the right platform [J]. Front Bioeng Biotechnol, 2016, 4: 12.

[44] LOU Y, GUO D, ZHANG H, et al. Effectiveness of mesenchymal stems cells cultured by hanging drop vs. conventional culturing on the repair of hypoxic-ischemic-damaged mouse brains, measured by stemness gene expression [J]. Open Life Sci, 2016, 11(1): 519-23.

[45] BRESLIN S, O'DRISCOLL L. Three-dimensional cell culture: the missing link in drug discovery [J]. Drug Discov Today, 2013, 18(5/6): 240-9.

[46] SHI W, KWON J, HUANG Y, et al. Facile tumor spheroids formation in large quantity with controllable size and high unifor-

mity [J]. Sci Rep, 2018, 8(1): 6837.
[47] BUMPERS H L, JANAGAMA D G, MANNE U, et al. Nano-magnetic levitation three-dimensional cultures of breast and colorectal cancers [J]. J Surg Res, 2015, 194(2): 319-26.
[48] ONBAS R, ARSLAN YILDIZ A. Fabrication of tunable 3D cellular structures in high volume using magnetic levitation guided assembly [J]. ACS Appl Bio Mater, 2021, 4(2): 1794-802.
[49] EDMONDSON R, BROGLIE J J, ADCOCK A F, et al. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors [J]. Assay Drug Dev Technol, 2014, 12(4): 207-18.
[50] CHAN Y H, LEE Y C, HUNG C Y, et al. Three-dimensional spheroid culture enhances multipotent differentiation and stemness capacities of human dental pulp-derived mesenchymal stem cells by modulating MAPK and NF-κB signaling pathways [J]. Stem Cell Rev Rep, 2021, 17(5): 1810-26.
[51] SOUZA G R, MOLINA J R, RAPHAEL R M, et al. Three-dimensional tissue culture based on magnetic cell levitation [J]. Nat Nanotechnol, 2010, 5(4): 291-6.
[52] TSENG H, BALAOING L R, GRIGORYAN B, et al. A three-dimensional co-culture model of the aortic valve using magnetic levitation [J]. Acta Biomater, 2014, 10(1): 173-82.
[53] TSENG H, DAQUINAG A C, SOUZA G R, et al. Three-dimensional magnetic levitation culture system simulating white adipose tissue [J]. Methods Mol Biol, 2018, 1773: 147-54.
[54] URBANCZYK M, ZBINDEN A, LAYLAND S L, et al. Controlled heterotypic pseudo-islet assembly of human beta-cells and human umbilical vein endothelial cells using magnetic levitation [J]. Tissue Eng Part A, 2020, 26(7/8): 387-99.
[55] NATÂNIA DE SOUZA-ARAÚJO C, RODRIGUES TONETTI C, CARDOSO M R, et al. Three-dimensional cell culture based on magnetic fields to assemble low-grade ovarian carcinoma cell aggregates containing lymphocytes [J]. Cells, 2020, 9(3): 635.
[56] GAITAN-SALVATELLA I, LOPEZ-VILLEGAS E O, GONZALEZ-ALVA P, et al. Case report: formation of 3D osteoblast spheroid under magnetic levitation for bone tissue engineering [J]. Front Mol Biosci, 2021, 8: 672518.
[57] KUO H J, MASLEN C L, KEENE D R, et al. Type VI collagen anchors endothelial basement membranes by interacting with type IV collagen [J]. J Biol Chem, 1997, 272(42): 26522-9.
[58] WANG S, SEKIGUCHI R, DALEY W P, et al. Patterned cell and matrix dynamics in branching morphogenesis [J]. J Cell Biol, 2017, 216(3): 559-70.
[59] HYNES R O. The extracellular matrix: not just pretty fibrils [J]. Science, 2009, 326(5957): 1216-9.
[60] HASTINGS J F, SKHINAS J N, FEY D, et al. The extracellular matrix as a key regulator of intracellular signalling networks [J]. Br J Pharmacol, 2019, 176(1): 82-92.
[61] LALWANI G, D'AGATI M, GOPALAN A, et al. Three-dimensional carbon nanotube scaffolds for long-term maintenance and expansion of human mesenchymal stem cells [J]. J Biomed Mater Res A, 2017, 105(7): 1927-39.
[62] 韦章澳, 徐凌寒, 吴子辰, 等. 无机非金属人工骨修复材料的体内应用[J]. 中国组织工程研究(WEI Z A, XU L H, WU Z C, et al. Application of inorganic nonmetallic artificial bone materials *in vivo* [J]. Chin J Tissue Eng Res), 2022, 26(16): 2584.
[63] 黄红茵, 蒋笑, 刘宏伟. 脱细胞外基质制备与应用的研究现状[J]. 感染、炎症、修复(HUANG H Y, JIANG X, LIU H W. Research status of preparation and application of acellular extracellular matrix [J]. Infect Inflamm Rep), 2021, 22(1): 49-53.
[64] LAI Y, SUN Y, SKINNER C M, et al. Reconstitution of marrow-derived extracellular matrix *ex vivo*: a robust culture system for expanding large-scale highly functional human mesenchymal stem cells [J]. Stem Cells Dev, 2010, 19(7): 1095-107.
[65] LI M, ZHANG T, JIANG J, et al. ECM coating modification generated by optimized decellularization process improves functional behavior of BMSCs [J]. Mater Sci Eng C Mater Biol Appl, 2019, 105: 110039.
[66] ZHU Y, ZHANG Q, WANG S, et al. Current advances in the development of decellularized plant extracellular matrix [J]. Front Bioeng Biotechnol, 2021, 9: 712262.
[67] JHALA D, RATHER H A, VASITA R. Extracellular matrix mimicking polycaprolactone-chitosan nanofibers promote stemness maintenance of mesenchymal stem cells via spheroid formation [J]. Biomed Mater, 2020, 15(3): 035011.
[68] DADASHPOUR M, PILEHVAR-SOLTANAHMADI Y, MOHAMMADI S A, et al. Watercress-based electrospun nanofibrous scaffolds enhance proliferation and stemness preservation of human adipose-derived stem cells [J]. Artif Cells Nanomed Biotechnol, 2018, 46(4): 819-30.
[69] AVOSSA J, HERWIG G, TONCELLI C, et al. Electrospinning based on benign solvents: current definitions, implications and strategies [J]. Green Chem, 2022, 24(6): 2347-75.
[70] HOARE T R, KOHANE D S. Hydrogels in drug delivery: progress and challenges [J]. Polymer, 2008, 49(8): 1993-2007.
[71] ZHENG S Y, DING H, QIAN J, et al. Metal-coordination complexes mediated physical hydrogels with high toughness, stick–slip tearing behavior, and good processability [J]. Macromolecules, 2016, 49(24): 9637-46.
[72] EROL O, PANTULA A, LIU W, et al. Transformer hydrogels: a review [J]. Adv Mater Technol, 2019, 4(4): 1900043.
[73] VALDOZ J C, JOHNSON B C, JACOBS D J, et al. The ECM: to scaffold, or not to scaffold, that is the question [J]. Int J Mol Sci, 2021, 22(23): 12690.
[74] CHIEN H W, FU S W, SHIH A Y, et al. Modulation of the stemness and osteogenic differentiation of human mesenchymal stem cells by controlling RGD concentrations of poly(carboxybetaine) hydrogel [J]. Biotechnol J, 2014, 9(12): 1613-23.
[75] GOH M C, TAE G. Mesenchymal stem cell-encapsulated cellulose nanofiber microbeads and enhanced biological activities by hyaluronic acid incorporation [J]. Carbohydr Polym, 2022, 280: 119026.
[76] LIN C, HE Y, FENG Q, et al. Self-renewal or quiescence? Orchestrating the fate of mesenchymal stem cells by matrix viscoelasticity via PI3K/Akt-CDK1 pathway [J]. Biomaterials, 2021, 279: 121235.
[77] REN Y, ZHANG H, WANG Y, et al. Hyaluronic acid hydrogel with adjustable stiffness for mesenchymal stem cell 3D culture via related molecular mechanisms to maintain stemness and induce cartilage differentiation [J]. ACS Appl Bio Mater, 2021, 4(3): 2601-13.
[78] SUN X, ZHANG H, HE J, et al. Adjustable hardness of hydrogel for promoting vascularization and maintaining stemness of stem cells in skin flap regeneration [J]. Appl Mater Today, 2018, 13:

54-63.

[79] PANDOLFI L, FURMAN N T, WANG X, et al. A nanofibrous electrospun patch to maintain human mesenchymal cell stemness [J]. J Mater Sci Mater Med, 2017, 28(3): 44.

[80] XING M, WANG X, WANG E, et al. Bone tissue engineering strategy based on the synergistic effects of silicon and strontium ions [J]. Acta Biomater, 2018, 72: 381-95.

[81] NOWWAROTE N, SUKARAWAN W, KANJANA K, et al. Interleukin 6 promotes an *in vitro* mineral deposition by stem cells isolated from human exfoliated deciduous teeth [J]. R Soc Open Sci, 2018, 5(10): 180864.

[82] FORRISTAL C E, WRIGHT K L, HANLEY N A, et al. Hypoxia inducible factors regulate pluripotency and proliferation in human embryonic stem cells cultured at reduced oxygen tensions [J]. Reproduction, 2010, 139(1): 85-97.

[83] FLOTTE T R, AFIONE S A, ZEITLIN P L. Adeno-associated virus vector gene expression occurs in nondividing cells in the absence of vector DNA integration [J]. Am J Respir Cell Mol Biol, 1994, 11(5): 517-21.

[84] CHEN J, HUANG Y, YANG J, et al. Multiple nanosecond pulsed electric fields stimulation with conductive poly(l-lactic acid)/carbon nanotubes films maintains the multipotency of mesenchymal stem cells during prolonged *in vitro* culture [J]. J Tissue Eng Regen Med, 2020, 14(8): 1136-48.

[85] MCMURRAY R J, GADEGAARD N, TSIMBOURI P M, et al. Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency [J]. Nat Mater, 2011, 10(8): 637-44.

[86] FRITH J E, THOMSON B, GENEVER P G. Dynamic three-dimensional culture methods enhance mesenchymal stem cell properties and increase therapeutic potential [J]. Tissue Eng Part C, 2010, 16(4): 735-49.

[87] SZOT C S, BUCHANAN C F, FREEMAN J W, et al. 3D *in vitro* bioengineered tumors based on collagen I hydrogels [J]. Biomaterials, 2011, 32(31): 7905-12.

[88] HUTCHINSON L, KIRK R. High drug attrition rates: where are we going wrong [J]? Nat Rev Clin Oncol, 2011, 8(4): 189-90.

[89] YAMADA K M, CUKIERMAN E. Modeling tissue morphogenesis and cancer in 3D [J]. Cell, 2007, 130(4): 601-10.

[90] XU F, BURG K J. Three-dimensional polymeric systems for cancer cell studies [J]. Cytotechnology, 2007, 54(3): 135-43.

[91] REGMI S, JEONG J H. Superiority of three-dimensional stem cell clusters over monolayer culture: an archetype to biological application [J]. Macromol Res, 2016, 24(12): 1037-46.

[92] HAN S, ZHAO Y, XIAO Z, et al. The three-dimensional collagen scaffold improves the stemness of rat bone marrow mesenchymal stem cells [J]. J Genet Genomics, 2012, 39(12): 633-41.

[93] QIAO Y, XU Z, YU Y, et al. Single cell derived spheres of umbilical cord mesenchymal stem cells enhance cell stemness properties, survival ability and therapeutic potential on liver failure [J]. Biomaterials, 2020, 227: 119573.

[94] DUGGAL S, FRØNSDAL K B, SZÖKE K, et al. Phenotype and gene expression of human mesenchymal stem cells in alginate scaffolds [J]. Tissue Eng Part A, 2009, 15(7): 1763-73.

[95] CHOI J R, YONG K W, WAN SAFWANI W K Z. Effect of hypoxia on human adipose-derived mesenchymal stem cells and its potential clinical applications [J]. Cell Mol Life Sci, 2017, 74(14): 2587-600.

[96] HUNG S C, POCHAMPALLY R R, CHEN S C, et al. Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis [J]. Stem Cells, 2007, 25(9): 2363-70.

[97] SUKARAWAN W, NOWWAROTE N, KERDPON P, et al. Effect of basic fibroblast growth factor on pluripotent marker expression and colony forming unit capacity of stem cells isolated from human exfoliated deciduous teeth [J]. Odontology, 2014, 102(2): 160-6.

[98] NEMEC S, KILIAN K A. Materials control of the epigenetics underlying cell plasticity [J]. Nat Rev Mater, 2020, 6(1): 69-83.

[99] NIU Y, ZHANG Y, HE W, et al. Engineering a microcarrier based on a polysaccharide-growth factor complex for enhancing the proliferation of mesenchymal stem cells [J]. Int J Biol Macromol, 2020, 155: 911-8.

[100] ZHANG L, XIONG N, LIU Y, et al. Biomimetic cell-adhesive ligand-functionalized peptide composite hydrogels maintain stemness of human amniotic mesenchymal stem cells [J]. Regen Biomater, 2021, 8(2): rbaa057.

[101] YUAN X, YUAN W, DING L, et al. Cell-adaptable dynamic hydrogel reinforced with stem cells improves the functional repair of spinal cord injury by alleviating neuroinflammation [J]. Biomaterials, 2021, 279: 121190.

[102] SWANSON W B, OMI M, WOODBURY S M, et al. Scaffold pore curvature influences MuSC fate through differential cellular organization and YAP/TAZ activity [J]. Int J Mol Sci, 2022, 23(9): 4499.

[103] CALIARI S R, VEGA S L, KWON M, et al. Dimensionality and spreading influence MSC YAP/TAZ signaling in hydrogel environments [J]. Biomaterials, 2016, 103: 314-23.

[104] SCHELLENBERG A, JOUSSEN S, MOSER K, et al. Matrix elasticity, replicative senescence and DNA methylation patterns of mesenchymal stem cells [J]. Biomaterials, 2014, 35(24): 6351-8.

[105] LÜ L, TANG Y, ZHANG P, et al. Biomaterial cues regulate epigenetic state and cell functions-a systematic review [J]. Tissue Eng Part B Rev, 2018, 24(2): 112-32.