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·综 述·

骨组织工程中脂肪干细胞成骨分化影响因素的研究进展

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［摘要］ 创伤、肿瘤、骨折并发症等造成的大段骨缺损是骨科领域的常见问题，传统治疗大段骨缺损采用的骨移植法由于材料来源有限、治疗周期长、并发症多等问题限制了自体或异体骨移植的应用和临床治疗效果。近年来，脂肪干细胞（ADSC）在骨组织工程中的应用为治疗大段骨缺损提供了新的思路和视角，很大程度上克服了传统骨移植的缺点与不足。多种因素在ADSC的成骨分化中发挥了重要作用，本文对骨组织工程中ADSC成骨分化的影响因素及其研究进展进行综述。

［关键词］ 骨组织工程；支架；脂肪干细胞；成骨；机械力；外泌体
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Influencing factors of osteogenic differentiation of adipose derived stem cells in bone tissue engineering: research progress

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［**Abstract**］ Large bone defects caused by trauma, tumor, fracture complications, etc. are common problems in the field of orthopaedics. Due to limited material source, long treatment period and various complications, the application and clinical effect of autologous or allogeneic bone transplantation are limited by the traditional bone grafting method for the treatment of large bone defects. In recent years, the application of adipose derived stem cells (ADSCs) in bone tissue engineering provides a new idea and perspective for the treatment of large bone defects, and to a large extent overcomes the shortcomings and deficiencies of traditional bone transplantation. Many factors play important roles in the osteogenic differentiation of ADSCs. The influencing factors and research progress of osteogenic differentiation of ADSCs in bone tissue engineering are reviewed in this paper.

［**Key words**］ bone tissue engineering; scaffold; adipose derived stem cell; osteogenesis; mechanical force; exosome
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随着现代社会的快速发展，由交通意外、建筑事故、骨肿瘤、手术并发症等因素造成的骨折不愈合、大段骨缺损等问题日益突出。传统骨缺损的治疗方法是骨移植，但自体移植骨的来源有限且会对机体造成二次损伤，异体移植骨的诱导性不足并且存在疾病传播风险[1]，这使得骨移植方法主要用于短节段骨缺损。当骨缺损长度超过自体修复临界状态时，常会出现骨吸收及不愈合[2]，组织工程的快速发展为治疗大段骨缺损及骨不愈合提供了新的思路和视角。作为组织工程三要素之一的脂肪干细胞（adipose derived stem cell，ADSC）来源于脂肪组织非脂肪细胞的基质血管成分，具有自我更新并分化为骨、软骨、脂肪等细胞的潜能[3]，相较骨髓间充质干细胞ADSC来源广泛、取材方便、免疫反应小，相较胚胎干细胞ADSC致瘤性低、无伦理学争议。近年来，随着ADSC在骨组织工程和再

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生医学中的应用，初步显现出治疗骨不连、大段骨缺损的潜在价值。支架材料、生物力学、细胞因子等多种因素在 ADSC 成骨分化中均发挥着重要作用，本文主要就骨组织工程中 ADSC 成骨分化的影响因素及研究进展进行综述。

1 生物因素对 ADSC 成骨分化的影响

1.1 细胞供体 早在 20 世纪 60 年代，Rodbell[4-5] 就从 SD 雄性大鼠脂肪组织中分离出成熟脂肪细胞与成脂前体细胞。哺乳动物脂肪组织通常分为棕色脂肪和白色脂肪，棕色脂肪主要作用是维持体温[6]，而白色脂肪主要储存能量[7]。通过傅立叶变换红外光谱检测发现，Wistar 大鼠皮下脂肪组织的蛋白含量及磷酸酶活性更高；相比于棕色脂肪，腹股沟皮下白色脂肪组织来源的细胞数量更多，且 Wistar 大鼠白色脂肪多分布于双侧腹股沟皮下及附睾 / 卵巢周围，棕色脂肪则分布于双侧肩胛骨间[3]。收集白色脂肪组织后可通过机械或酶解法分离出 ADSC，其中酶解法效率是机械法的 1 000 倍[8]。ADSC 的增殖速度与成骨分化能力随供体年龄的增加而降低[9]，相同诱导条件下鼠和人来源的 ADSC 成骨分化能力优于兔 ADSC[10]。细胞活力和增殖能力会随传代次数的增加而减弱，用于组织工程研究的白色 ADSC 通常选用第 2～5 代细胞[11]。

1.2 外泌体与细胞外基质 外泌体是细胞间传递生物信息的纳米级囊泡[12]，负载着调控多种细胞生物学行为的蛋白质、miRNA 和 DNA 等小分子物质[13-14]。魏松乔等[15]发现经成骨诱导后，ADSC 来源的外泌体可显著促进支架上 ADSC 的成骨分化。除提前收集外泌体外，还可将成骨细胞与 ADSC 共培养于支架，通过成骨细胞旁分泌作用实时促进 ADSCs 的成骨分化[16]。此外，脂肪前体细胞来源的外泌体通过 TGF-β 途径也可促进脂肪前体细胞的成骨分化[17]。天然细胞外基质为细胞提供了黏附、增殖和分化场所，相同条件下 ADSC 来源的细胞外基质中纤维连接蛋白和胶原含量均多于成纤维细胞[18]。细胞膜片技术可实现将 ADSC 与其外基质完整地连结在一起，应用于支架材料能更好地促进骨愈合[19]，但由于天然细胞外基质的机械性能差和容易降解，限制了其广泛应用。水凝胶作为一种合成材料，吸水膨胀性好，结构与软组织相似，能很好地弥补天然细胞外基质的不足[20]。

1.3 RNA 与激素 随着现代分子生物学的快速发展，越来越多的证据表明非编码 RNA 如 miRNA、lncRNA、环状 RNA（circular RNA，circRNA）密切参与了机体生理或病理状态表观遗传学的基因调控，其中 miRNA 调控细胞中近 30% 的基因表达[21]，lncRNA 通过多种机制影响基因转录、RNA 剪接调控、mRNA 降解、翻译调控等过程，circRNA 除调节细胞代谢外还可作为某些疾病的生物标志物[22-23]。非编码 RNA 主要通过 Wnt/β-catenin、NF-κB 受体活化因子（receptor activator of nuclear factor κB，RANK）/NF-κB 受体活化因子配体（receptor activator of nuclear factor κB ligand，RANKL）2 条信号通路分别调节成骨细胞与破骨细胞活动，最终维持骨组织的稳态[23]，如过表达 miRNA-130a-3p 部分通过去乙酰化酶 7（sirtuin 7，SIRT7）/Wnt/β-catenin 通路增加 ADSC 成骨分化但同时抑制其增殖[24]。可以通过基因数据库与生物信息学技术分析 ADSC 成骨分化过程中差异表达的 RNA，进而筛选出能促进其成骨分化的小分子。此外，体内激素水平对 ADSC 的成骨分化也有一定影响。2 型糖尿病可降低 ADSC 的活性，表达的成骨分化标志物多于胰岛素抵抗患者来源的 ADSC[25]，胰高血糖素样肽通过 Wnt/ 糖原合成酶激酶 3β（glycogen synthase kinase 3β，GSK-3β）/β-catenin 通路促进了人 ADSCs 的成骨分化而抑制了其成脂分化[26]，甲状旁腺激素可促进 ADSC 的成骨分化[27]。

2 非生物因素对 ADSC 成骨分化的影响

2.1 材料性能 用于组织工程的支架应具备良好的生物相容性，即细胞能在支架上黏附、增殖，且几乎不会引起宿主免疫排斥反应。此外，随着成骨细胞数量逐渐增多，支架材料能按一定的速率降解，为无机物和新生血管拓展提供空间[28]。传统的无机物支架机械强度大，但不可降解，且韧性和骨诱导性差[29]，植入体内后往往需要二次手术取出。在有机高分子支架与可降解生物聚合物中添加一定量的无机物能增强支架的机械强度、骨传导性和骨诱导性，更好地模拟出骨组织的结构和功能[30]。随着现代医学科技的快速发展，松质骨的形态特征可在体外支架材料上进行重建，具有不同拓扑结构表面的支架对 ADSC 的影响不同。岛状结构表面促进 ADSC 成骨分化的效果最好，而蜂窝

状结构表面会阻碍其增殖与分化[31]；针形结构表面有利于细胞早期黏附并促进成骨分化[32]。

2.2　材料血管化　正常骨组织依靠中央管获取营养，因此大段骨缺损在移植支架的同时保证血供非常重要，支架内部的孔隙要为细胞增殖与新生血管提供足够的空间。将ADSC与血管内皮细胞同时种植于支架上可改善支架内部的血供，从而更好地促进细胞生长[33]。此外，还可先将血管内皮细胞种植于支架，或将支架上的ADSC通过血管内皮生长因子（vascular endothelial growth factor，VEGF）诱导分化为血管内皮细胞[34]制备出预血管化材料后再种植ADSC，这样也能较好地促进ADSC成骨分化[35]。但上述共培养细胞过程与所需设备条件比较复杂，目前已有通过支架释放促成骨与血管化生长因子[36]和微量元素[37]的研究。由于支架负载的VEGF和骨形态发生蛋白（bone morphogenetic protein，BMP）2释放速率与剂量较难控制，因此研发了能同时促进干细胞成骨分化和血管分化的生物材料，如支架药载辛伐他汀被证明具有良好的促血管生成和成骨作用[30]。除了负载药物，基因递送如过表达缺氧诱导因子（hypoxia-inducible factor，HIF）也能促进支架早期血管化[38]。

2.3　材料氧供　氧气对细胞生长代谢至关重要，改进材料成分能释放氧气，增加细胞外基质中的氧含量。骨组织的生理氧含量为5%～12%[39]。材料释放的氧水平对干细胞分化具有不同的作用，低氧状态下能促进细胞的自我更新但同时会抑制其成骨分化[40]，在一定范围内高氧水平可促进ADSC的成骨分化，而低氧水平则促进其成软骨分化[41]。罗凯等[42]发现，含10%全氟三丁胺的材料在缺氧条件下可促进兔ADSC的增殖、黏附及成骨分化。目前认为HIF参与调节氧水平对细胞分化影响的过程，在高氧水平下HIF被降解且其合成被抑制，而在低氧水平下HIF信号通路被激活[43]。Xia等[44]发现负载姜黄素可增强3D打印水凝胶材料的抗氧化性，并且能降低负载人ADSC的氧化凋亡，最终提高支架复合物的体内置入成功率。

2.4　流体剪切力　细胞外液约占体重的20%，为细胞的生长代谢提供了物质交换场所，同时细胞外液在细胞表面形成流体静水压，梯度静水压驱动体内组织液的不断流动使得细胞表面受到相应的流体剪切力。Elashry等[45]发现流体剪切力可促进支架材料复合体上ADSC中Runt相关转录因子2（Runt-related transcription factor 2，RUNX2）、碱性磷酸酶（alkaline phosphatase，ALP）等成骨基因的表达。运动状态也能改变流体剪切力，肌肉收缩时血管被暂时挤压排空导致静水压升高，而舒张时由于血管的负压回流又使其降低[46]，综合结果导致骨组织内的静脉与静水压变化，从而造成骨髓腔内的压力波动。Tuin等[47]发现对叶片状支架上的人ADSC施加波动流体剪切力，可显著促进成骨早期标志物RUNX2的表达。此外，液体的流速不同对干细胞成骨分化的影响也不同，在一定范围内低流速的流体剪切力更能促进干细胞的成骨分化，而流速过高则会抑制成骨分化[48]。

2.5　微重力　随着载人航天技术的飞速发展，宇航员在太空中能停留的时间越来越久，但同时在太空微重力环境下宇航员的骨密度以每个月1%的速率流失，并且呈时间累积关系[49-50]。微重力环境下干细胞的成骨分化受到抑制、成脂分化增强[51]，同时复合水凝胶支架上ADSC的增殖能力和成软骨分化均增强[52]。微重力环境下破骨细胞活性及抗酒石酸酸性磷酸酶（tartrate resistant acid phosphatase，TRAP）、组织蛋白酶K、基质金属蛋白酶9（matrix metalloproteinase 9，MMP-9）表达增强[53]，其中自噬作用参与增强破骨细胞分化过程[54]。Mashiko等[55]在微重力条件下将人ADSC与聚苯乙烯和胶原微球结合，1周后检测细胞多能性标志物发现这种培养方式可以更好地促进间充质干细胞的增殖与多向分化。骨折患者由于长期卧床，身体负荷被完全或部分分散，处于类似微重力的状态而造成骨质流失[56]。针对微重力引起的骨质流失，适当强度的对抗运动及补充适量的营养有助于减少骨质疏松的发生[57]。然而Zhao等[58]发现，微重力环境下同时负载BMP2/7的颗粒复合体能更好地促进ADSC成骨分化，这为拮抗微重力引起的骨质疏松提供了新视角。

2.6　机械应力　细胞外环境中的应力通常以固体、液体和气体形式直接或间接作用于细胞，通过细胞外基质－整合素－细胞骨架系统、力敏感性离子通道、G蛋白偶联受体激酶系统途径将细胞外基质的力学信号转变为细胞内生物信号[59-60]。Gabbay等[61]将拉力与压力作用于人ADSC凝胶支架，发现拉力或压力均能促进成骨基因表达，且拉

力复合压力比单纯拉力刺激更早地促进成骨基因表达。早期应力可显著促进细胞成骨分化，但持续时间过长将对新生血管产生损害及阻碍骨折愈合过程[62]。Virjula 等[63]的研究显示，对人 ADSC 施加循环等轴拉伸力可延缓 ADSC 的增殖并促进其成骨分化，拉伸还减小了细胞大小、增强了局部粘连和肌动蛋白细胞骨架。研究表明一定范围内低幅度高频率的振动能促进人 ADSC 成骨分化而抑制其成脂分化，并且通过促进细胞增殖和细胞骨架重塑而增强细胞机械传导作用[64]。总之，最佳的机械刺激参数还需进一步探索。

3 小 结

组织工程与再生医学的快速发展为大段骨缺损与骨不连治疗提供了新方法，作为组织工程三要素之一的 ADSC 因其来源广泛、取材方便、无伦理争议等诸多优势近年得到越来越多的关注与研究。近年新兴可降解材料的骨诱导性和传导性均较好，且移植体内后无须二次手术取出，因此也备受关注。通过改变材料的性质和结构，同时对细胞支架复合体施加外界因素能促进 ADSC 成骨分化，但其具体机制及如何以最佳的形式和参数增强其成骨分化仍有待进一步研究。

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