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综 述

脂肪干细胞的共培养及其分化应用概述

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摘 要: 干细胞在体外特定培养条件下可以被诱导分化成具有不同体细胞表型的细胞。除了通过不同培养条件进行体外诱导分化的方法外，用成熟体细胞与干细胞共培养同样可以诱导干细胞定向分化。以下首先简述了脂肪干细胞(Adipose-derived stem cells，ADSCs) 的来源及其标志，然后重点就 ADSCs 的不同培养方法、诱导分化及最新的临床应用进行阐述，包括药物及化学诱导培养、体细胞与 ADSCs 二维、三维共培养等，最后提出 ADSCs 的问题所在并对此技术进行展望。

关键词: 脂肪干细胞，共培养，流式细胞仪，组织工程，再生医学

Progress in co-culture and differentiation in adipose-derived stem cells: a review

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Abstract: Stem cells can be differentiated into many kinds of somatic cells under defined culture conditions. In addition, the homing possess can be partially imitated by co-culture of stem cells with mature somatic cells. Regarding the importance of clinical application of adipose-derived stem cells (ADSCs), our review first introduced the sources and signs of ADSCs, and then the current knowledge of ADSCs co-culture technology, including drug and chemical induced culture, two-dimensional (2D) and three-dimensional (3D) co-culture, mechanisms of ADSCs differentiation, and application development in recent years in details. Finally, we also addressed prospects of ADSCs.

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Keywords: adipose-derived stem cells (ADSCs), co-culture, flow cytometry, tissue engineering, regenerative medicine

目前，组织工程与基因治疗研究较多的靶细胞是造血干细胞 (Hematopoietic stem cells，HSCs) 和骨髓间充质干细胞 (Bone mesenchymal stem cells，BMSCs)。这两种细胞都具备较强的体外增殖能力和分化能力。但它们主要分布在骨髓，由于成人骨髓量有限，而且抽取具有创伤及严重的并发症等，故其应用受到限制。自 Zuk 等[1]首先报道成功分离培养人的脂肪干细胞以来，ADSCs 逐渐成为各国科学家研究的热点。来源于人及各种动物的 ADSCs 的培养也各异。本文就 ADSCs 的药物化学诱导培养、共培养及其最新应用等方面作一综述。

1 ADSCs 的来源及其标志

Zuk 等[1]从人体脂肪组织中分离培养出成纤维样细胞群，能在体外稳定扩增，免疫荧光和流式细胞仪分析发现，这些细胞中大部分是中胚层，在一定条件下，可以向脂肪、软骨、肌肉和成骨细胞分化，称这些细胞为脂肪干细胞。细胞周期分析表明 ADSCs G0/G1 期 79.1%，S 期 19.7%，G2/M 期 1.3%，大部分细胞处于 G0/G1 期，仅有少数细胞处于活跃的增殖期，说明只有机体受到损伤等应激情况时，ADSCs 的自我更新方式才会发生变化。

有关 ADSCs 特异性表面标志至今仍无统一认识，不同实验室研究结果不尽相同，这种差异可能与细胞分离、培养和纯化方法存在差异有关。流式细胞技术和免疫组织化学检测发现其中活化淋巴细胞粘附分子 (CD166)、血管细胞粘附分子 (CD106)、Thy21 (CD90)、5′-外切核酸酶 (CD73)、CD63、透明质酸盐 (CD44)、整合素 β1(C29) 等起初表达较低，随着传代其表达显著增加；而干细胞相关表面标志 CD34 的表达一直很高。ADSCs 一般不表达 E2 选择素 (CD62)、神经细胞粘附分子 (CD56) 等[2-7]。如用这些标志对 ADSCs 进行有效分选，可以得到较纯的 ADSCs，将有助于对 ADSCs 的进一步研究[8] (表 1)。

表 1 ADSCs 与 BMSCs 的表面标志比较[8-13]

Table 1 Surface markers of ADSCs and BMSCs[8-13]

Surface markers	ADSCs expression	BMSCs expression
CD3	−	−
CD9	+	+
CD10	+	+
CD11	−	+
CD13	+	+
CD14	−	−
CD18	−	−
CD29	+	+
CD31	−	−
CD34	−	−
CD44	+	+
CD45	−	−
CD49d	+	−
CD49e	+	+
CD50	−	−
CD51	+	+/−
CD54	+	+
CD55	+	+
CD59	+	+
CD61	+/−	+/−
CD62L	−	−
CD63	+	+
CD71	+	+
CD73	+	+
CD90	+	+
CD95L	−	−
CD105	+	+
CD106	−	+
CD117	+	+
CD133	−	+
CD146	+	+
CD166	+	+
STRO-1	+/−	+
HLA-ABC	+	+
HLA-DR	−	−
Vimentin	+	+
Collagen type I	+	+
Collagen type Ⅱ	+	+
Osteopontin	+	+
Osteonectin	+	+

2 ADSCs 的培养与分化

2.1 ADSCs 的培养

ADSCs 可来源于人类、猴、犬、牛、猪、兔、豚鼠、大鼠、小鼠等，其培养方法大同小异。取腹股沟平行切下脂肪组织或吸脂手术的脂肪抽吸物，用 PBS 或者 D-Hanks 液冲洗，将脂肪组织剪成 1 mm×1 mm×1 mm 大小，加入 0.1%的Ⅰ型胶原酶，37 ℃搅拌 40~60 min，1 000× g 离心 10 min，去上清，沉淀用 PBS 或者 D-Hanks 液洗，10%胎牛血清的 DMEM (低糖，2 mmol/L 谷氨酰胺，双抗)稀释，将细胞收集培养。24 h 后换液，3 d 换液 1 次，待细胞长满 80%用 0.25%胰酶消化传代培养(图 1)[13,18-21]。

2.2 ADSCs 的分化潜能

ADSCs 具有多项分化潜能，在不同的药物及化学诱导剂条件下可以分化成成骨细胞、软骨细胞、脂肪细胞、神经细胞等不同的细胞。如图 2、表 2 所示。

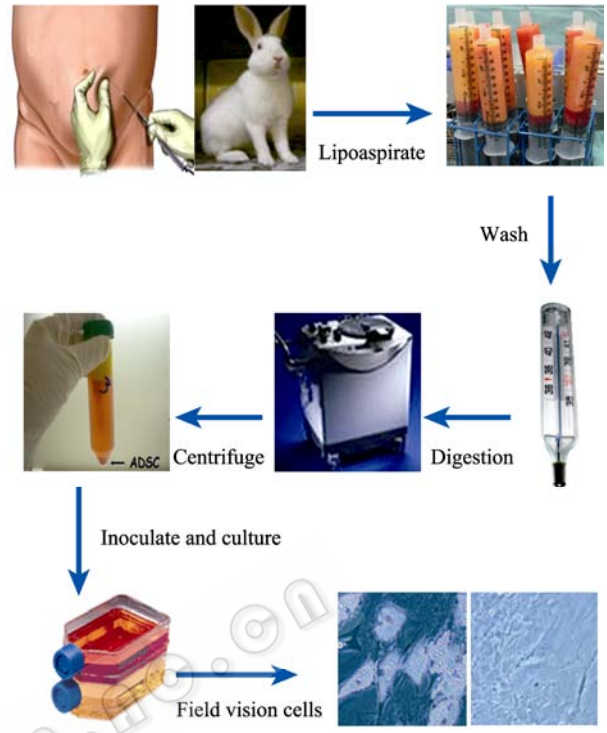

图 1 ADSCs 的取材及培养步骤

Fig. 1 Processing of lipoaspirate and isolation of adipose-derived stem cells.

表 2 ADSCs 的分化诱导条件[13,20-25]

Table 2 Conditions of ADSCs differentiated to special cell lineages[13,20-25]

Cell types	Culture medium	Serum	Add ingredients
Osteoplast	DMEM	10% FBS	0.1 μmol/L dexamethasone+50 μmol/L 2-ascorbic acid phosphate salt+ 10 mmol/L β-glyceroposphate
	DMEM	10% FBS	0.1 μmol/L dexamethasone+50 μmol/L 2-ascorbic acid phosphate salt
Chondrocyte	DMEM	1% FBS	6.25 mg/L insulin+0.01 mg/L TGF-β1+50 nmol/L 2-ascorbic acid phosphate salt
	DMEM	1% FBS	6.25 mg/L insulin+0.01 mg/L TGF-β1+6.25 mg/L transferrin
Adipose cells	DMEM/F12	—	100 μmol/L Vc+0.85 μmol/L Insulin+20 nmo1/L sodium selenite+ 0.2 nmo1/L 3, 5, 3′-triiodothyronine (T3)+1 μmol/L rosiglitazone+l μmol/L dexamethasone+0.1 mmol/L isobutyl−methylxanthine (IBMX)
	DMEM	10% FBS	0.5 mmol/L isobutyl-methylxanthine (IBMX)+1 μmol/L dexamethasone+ 10 μmol/L insulin+200 μmol/L indometacin
Nerve cells	DMEM	—	200 μmol/L butylated hydroxyanisole (BHA)+5 μmol/L KCl+2 μmo/L valproic acid+10 μmol/L forskolin+l μmol/L hydrocortisone+5 mg/L insulin
	A-MEM	10% FBS	10 μmol/L 5-azacitidine+50 ng/L NGF+l0 ng/L BDGF+5 ng/L bFGF
Myocardial cells	DMEM	10% FBS	Cardiomyocyte extract
	RPMI-1640	15% FBS	9 μmol/L 5-azacitidine
Skeletal muscle cells	DMEM	10% FBS+5% horse serum	0.1 μmol/L dexamethasone+50 μmol/L hydrocortisone

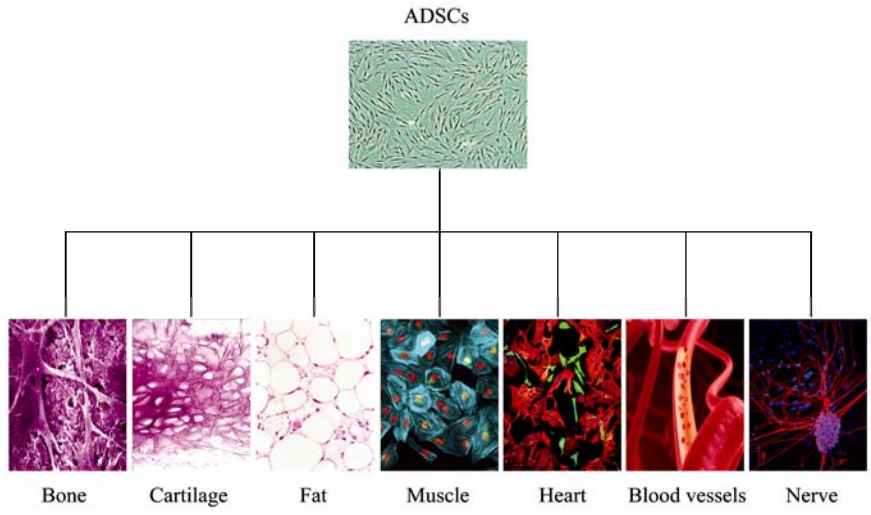

图 2　ADSCs 多向分化潜能[14-17]

Fig. 2　Multiple lineage differentiation potential of ADSCs. Under specific and controlled culture conditions, ADSCs can be induced to express the phenotypic characteristics of chondrocytes, osteoblast, adipocytes, or neurons, and so on[14-17].

3　ADSCs 的共培养

3.1　ADSCs 共培养介绍

ADSCs 除了通过不同的药物化学物质进行体外诱导分化的方法外，也可以采用成熟体细胞或细胞支架与其共培养的方式诱导其定向分化。与试剂诱导相对，共培养方法亦称为生物诱导。通常采用的共培养方式有单层共培养 (Monolayer co-culture)、悬浮培养 (Suspension culture)、间接共培养 (亦称分层渗透培养) (Transwell) 细胞团块培养 (Cell pellet)、细胞层培养 (Cell sheet)、三维支架培养 (Three-dimensional cell culture，TDCC)。其中单层培养、悬浮培养、间接共培养为二维培养，细胞团块培养、细胞层培养、三维细胞支架培养为三维培养 (图 3)。

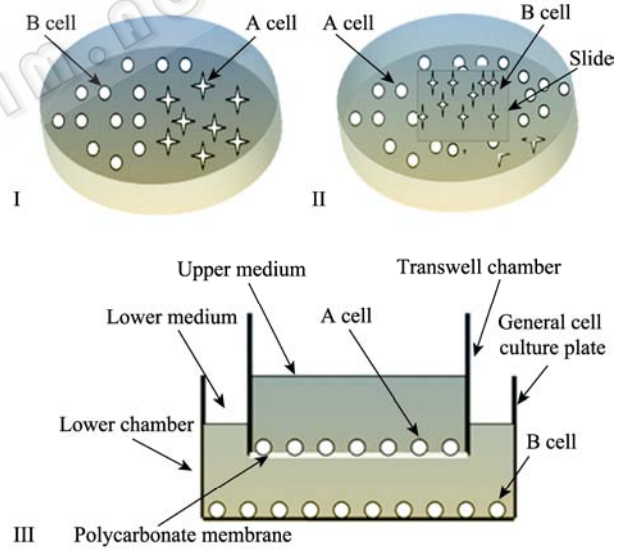

图 3　细胞共培养模型示意图

Fig. 3　Schematic diagram of cell co-culture model. (Ⅰ) A cell and B cell were co-cultured directly. (Ⅱ) A cell and B cell were co-cultured indirectly. (Ⅲ) Transwell chamber.

3.2　ADSCs 的二维培养

Zhang 等[26]用新西兰大白兔的 ADSCs 与软骨细胞共培养，2 周后成功地将 ADSCs 诱导成软骨样细胞。Cousin 等[27]通过将 ADSCs 与胰腺肿瘤细胞体外共培养，成功地利用 ADSCs 介导的肿瘤细胞凋亡，并且在体内实验和体外实验都得到了证实，预测 ADSCs 可以作为治疗胰腺瘤的细胞替代疗法。张勇等[28]将 ADSCs 与病理状态下软骨细胞共培养后，ADSC 可以被诱导成软骨样细胞，且发现高浓度血清三维培养可以增强这种诱导作用。Lei 等[29]通过 Transwell 共培养的方法检测了 ADSCs 对鼓膜成纤维细胞增殖的影响，通过 Transwell 细胞迁移实验检

测 ADSCs 对鼓膜成纤维细胞迁移速度的影响，激光共聚焦显微镜下评估各项检测指标。结果发现 ADSCs 可能通过旁分泌作用促进鼓膜成纤维细胞增殖和迁移，将有利于鼓膜纤维层的修复，为 ADSCs 最终应用于临床治疗鼓膜穿孔提供了有意义的实验依据。Zhang 等[30]将 ADSCs 与 pcDNA3.0-FGF10 重组质粒转染人胚来源的 MSC (pcDNA3.0-FGF10-MSC) 共培养，将其分别种植在半透膜细胞培养池 (Cell culture inserts) 多孔膜的外侧和内侧，通过监测转录因子 C/EBP-α、C/EBP-β 和 PPAR-γ 的表达情况以及裸鼠背部皮下混合注射 ADSCs 与 pcDNA3.0-FGF10-MSC 的体内实验来研究 FGF10 的促成脂作用，结果发现随着共培养时间的延长，半透膜细胞培养池内的 ADSCs 形态逐渐发生变化，胞内脂滴随着时间的延长而增大、增多，对照组的 ADSCs 没有明显改变，油红 O 染色也得到进一步证实，通过共培养的方法第一次从功能获得的角度证明了 FGF10 对 ADSCs 的促成熟作用。Li 等[31]将 ADSCs 与血旺细胞 (Schwann cells，SCs) 通过 Transwell 共培养，用免疫分析、Western blotting、RT-PCR 等分别检测 ADSCs 的形态、蛋白表达、基因等，发现 ADSCs 与 SCs 共培养，SCs 可以将其诱导向神经细胞分化，共培养 7 d 后开始显现，可以稳定保持 14 d。

3.3 ADSCs 的三维培养

Tang 等[32]用成年兔皮下脂肪分离培养脂肪干细胞，与自体脱钙骨基质复合后三维培养，细胞在脱钙骨基质表面和孔隙内黏附生长良好，并能继续增殖和分泌胞外基质。结果提示 ADSCs 与脱钙骨基质复合后三维培养增殖良好。Hao 等[33]利用Ⅰ型胶原凝胶均匀包埋 ADSCs 后将其与聚乳酸聚乙醇酸-β-磷酸三钙支架 (PLGA-β-TCP) 材料复合，以增加细胞在材料表面的附着，有效改善 ADSCs 与支架材料的复合方式，并观察其异位成骨情况，结果发现通过应用Ⅰ型胶原凝胶来实现脂肪干细胞与 PLGA-β-TCP 多孔支架材料的均匀复合，能够有效促进 ADSCs 在材料孔隙中的成骨分化及均质骨组织形成。Tian 等[34]用 3 月龄的日本大耳兔的 ADSCs 在体外壳聚糖-藻酸盐凝胶三维支架诱导培养下，成功分化成髓核样细胞，产生与髓核样细胞相同的胞外基质，且发现低氧状态下诱导效果较好。Nieto-Aguilar 等[35]将人的 ADSCs 在三维新型纤维蛋白琼脂糖脚手架上进行诱导分化，用组织化学、免疫荧光分析等技术证明三维纤维蛋白琼脂糖生物材料与 ADSCs 有高度的生物相容性，完全能够支持 ADSCs 向软骨、成骨、成脂、神经系分化。

4 ADSCs 的应用

4.1 ADSCs 在动物模型上的应用

ADSCs 在各疾病动物模型上用来干预治疗糖尿病、肝脏损伤修复、膀胱重建、2 型糖尿病阳痿、心肌梗死、脑梗、认知功能障碍、中风、椎间盘修复、胶质母细胞瘤治疗、骨缺损、创伤后血管新生、风湿性关节炎、肌腱修复、唇腭裂、心力衰竭、结肠炎、尿失禁等[36-48]。

4.1.1 *ADSCs 在心血管疾病动物模型中的应用*

西班牙学者 Perán 等[38]首次将 ADSCs 转变为心肌细胞，既重新编程了成熟的干细胞，又能改善心脏病的治疗。Zhang 等[39]将 ADSCs 与纤维蛋白胶复合后注射到心肌梗塞大鼠的左心室壁上，术后 4 周就发现大鼠的心脏功能相较于单独使用 ADSCs 组的大鼠有明显提高 ($P<0.01$)，提示联合 ADSCs 与纤维蛋白胶复合在临床组织工程治疗心肌梗塞上会有很大的前景。

4.1.2 *ADSCs 在组织工程动物疾病模型中的应用*

McIntosh 等[40]利用 ADSCs 促进大鼠脊柱融合，Zhu 等[41]用 ADSCs 接种到兔膀胱无细胞基质 (Bladder acellular matrix grafts，BAMGs) 中，24 周后各种指标数据表明成功地实现了兔膀胱的重建。Cui 等[42]将 ADSCs 作为种子细胞放于网状支架中，成功地修复了狗的颅骨损伤，为临床治疗颅骨损伤

提供了新的途径，Jing 等[43]提出可以利用 ADSCs 的骨分化特性来完整地修复牙齿，免除老年人牙齿缺失之苦。

4.1.3 *ADSCs 在代谢性疾病动物模型中的应用*

Ohmura 等[44]将 ADSCs 和胰岛细胞一起移植到 1 型糖尿病 C57BL/6J 小鼠中，结果显示胰岛细胞的存活率及其分泌胰岛素的功能都大大提高。Lin 等[45]将人或大鼠的 ADSCs 移植到 1 型糖尿病大鼠模型的肾小囊内后，实验动物出现血糖降低、葡萄糖耐量提高、皮肤光滑、白内障减少等表现，故表达胰十二指肠同源盒基因-1 (Pancreatic duodenal homeobox 1 gene，*Pdx1*) 的 ADSCs 可用于治疗糖尿病。

4.1.4 *ADSCs 在勃起功能障碍疾病动物模型中的应用*

美国加州大学医学院的 Garcia 等[46]向肥胖的 2 型糖尿病 ZDF 阳痿大鼠阴茎中注射大鼠自体的 ADSCs，注射 3 周后就发现 ADSCs 能够很好地存活，阴茎背根内神经源型一氧化氮合酶 (Neuronal nitric oxide synthase，nNOS) 也明显增加，并且明显改善了糖尿病阳痿大鼠的勃起功能障碍，预测 ADSCs 用来治疗糖尿病阳痿是一种很有前景的疗法。Huang 等[47]将 ADSCs 注射入高脂血症大鼠 (Hyperlipidemic rats，HR) 阴茎海绵体后，其 nNOS 的表达及海绵体压力 (Intracavernous pressure，ICP) 都较对照组高。Albersen 等[48]也证明 ADSCs 能有效改善神经性勃起功能障碍 (Neurogenic erectile dysfunction，NED) 大鼠的阴茎勃起情况。

4.2 ADSCs 在临床上的应用

目前，ADSCs 除了在各个动物模型上的应用取得了一定的成效，而且已经开始在临床上大量应用于组织工程、心血管疾病、代谢性疾病、脑病[49-58]等 (表 3)。

4.2.1 *ADSCs 在临床心血管疾病上的应用*

来自荷兰埃拉斯莫斯大学的杜克斯博士的科研小组[51]把心脏病人腹部脂肪的 ADSCs 注射进他们的心脏以后，减少了心脏的损伤、同时也增加了血流，干细胞注射进心脏以后经过 6 个月，病人心脏接受带氧血液的能力提高，心脏左心室送出的血液也增加了 3.5 倍，SPECT 显像证明接受 ADSCs 注射的病人心泵的能力增加了 5.7%，核磁扫描也显示患者的平均心肌疤痕面积由原来的 31.6%下降到 15.4%，这一研究成果发布在 2010 年美国心脏协会科学年会上。

4.2.2 *ADSCs 在临床组织工程上的应用*

ADSCs 在组织修复过程中可以调节周围组织中的生长激素和细胞因子如 VEGF，ICG-1 等防治细胞凋亡[52]，Kim 等[53-54]也证明 ADSCs 可以分泌各种皮肤生长因子如纤维细胞生长因子 (Basic fibroblast growth factor，bFGF) 等来促进成纤维细胞繁殖，并具有抗光老化、抗氧化、抗皱、抗紫外辐射等作用。Garcia-Olmo 等[55]研究小组用 ADSCs 来治疗病人复杂性肛瘘已经进入到 II 期临床实验。Yoshimura 等[56]用人的 ADSCs 移植入 15 名乳房萎缩、大小不一或需要隆胸的患者乳房中，3D 检查显示 ADSCs 的存活率达到 40%~80%，12 个月后，左右乳房大小均衡对称，乳房 X 光检测，被修复的乳房自然柔软完全无钙化，无囊肿现象，无明显的注射疤痕。

表 3 ADSCs 目前的临床研究

Table 3 Ongoing human clinical trials of adipose-derived stem cells

Status	Study	Phase	Sponsor	Condition	Intervention
Recruiting	Autologous adipose-derived stem cell transplantation in patients with lipodystrophy	Phase I	Irmandade santa casa de misericórdia de porto alegre	Lipodystrophy	Procedure: autologous transplantation of liposuction material enriched with adipose- derived stem cells
Active, not recruiting	Safety and efficacy of autologous adipose-derived stem cell transplantation in type 2 diabetics	Phase I Phase II	Adistem Ltd	Type 2 diabetes mellit-us	Procdure: autologous adipose-derived stem cells

续表 3

Completed	Safety and efficacy study of autologous cultured adipose -derived stem cells for the crohn's fistula	Phase I	Anterogen Co., Ltd.	Crohn's fistula	Drug: adipoplus
Recruiting	Safety study of autologous cultured adipose-derived stem cells for the fecal incontinence	Phase I	Anterogen Co., Ltd.	Fecal incontinence	Biological: ANT-SM
Recruiting	Safety and efficacy of autologous adipose-derived stem cell transplantation in patients with type 1 diabetes	Phase I Phase II	Adistem Ltd	Type 1 diabetes mellitus	Procdure: autologous adipose-derived stem cells
Recruiting	Safety and efficacy study of autologous cultured adipose -derived stem cells for the crohn's fistula	Phase II	Anterogen Co., Ltd.	Crohn's fistula	Drug: adipoplus
Active, not recruiting	Randomized clinical trial of adipose-derived stem cells in the treatment of Pts with ST-elevation myocardial infarction	Phase I	Cytori therapeutics	Myocardial infarction; coronary arteriosclerosis; cardiovascular disease; coronary disease	Drug: injection of ADRCs; other: injection of placebo
Recruiting	Development of bone grafts using adipose derived stem cells and different scaffolds	NA	University of zurich	Overweight	Procedure: cell culture
Recruiting	Allogenic stem cells derived from lipoaspirates for the treatment of recto-vaginal fistulas associated to crohn`s disease (ALOREVA)	Phase I Phase II	Fundacion para la investigacion biomedica del hospital universitario la paz	Rectovaginal fistula; crohn disease	Drug: expanded allogenic adipose-derived adult stem cells
Completed	Efficacy and safety of adipose stem cells to treat complex perianal fistulas not associated to crohn´s disease	Phase III	Cellerix	Anal fistula	Drug: ASCs (Cx401, company code); drug: fibrin adhesive
Active, not recruiting	A randomized clinical trial of adipose-derived stem cells in treatment of non revascularizable ischemic myocardium	Phase I	Cytori therapeutics	Ischemic heart disease; conary arteriosclerosis; cardiovascular disease; coronary disease; cornary artery disease	Other: direct injection of ADRCs into the left ventricle; other: direct injection of placebo into the left ventricle
Recruiting	Long-term safety and efficacy of adipose-derived stem cells to treat complex perianal fistulas in patients participating in the FATT-1 randomized controlled trial	NA	Cellerix	Complex perianal fistula	Drug: ASCs; drug: fibrin glue
Active, not recruiting	Study of autologous fat enhanced w/ regenerative cells transplanted to reconstruct breast deformities after lumpectomy	Phase IV	Cytori therapeutics	Breast neoplasms;carcinoma,ductal,breast;mammoplasty ; mastectomy, segmental, lumpectomy, breast reconstruction	Procedure: ADRC-enhanced autologous fat transplant
Active, not recruiting	Autologous stem cells derived from lipoaspirates for the non-surgical treatment of complex perianal fistula	Phase II	Cellerix	Anal fistula	Procedure:non-surgical autologous implant of ASCs
Recruiting	A cell dream for adipose cell derived regenerative endothelial angiogenic medicine	Phase I Phase II	University hospital, toulouse	Peripheral vascular diseases; cardiovascular diseases	Other: drug: expanded autologous ASC-s
Recruiting	Treatment of fistulous crohn's disease by implant of autologous mesenchymal stem cells derived from adipose tissue	Phase I Phase II	Instituto cientificoy Tecnologico de navarra, universidad de navarra	Crohn disease	Other: autologous mesenchymal stem cells

Sources: www. clinicaltrials. gov. Abbreviations: ASCs, adipose-derived stem cells; NA, no available information.

4.2.3 *ADSCs 在临床代谢性疾病上的应用*

最近的临床研究显示[57]，肌内注射 ADSCs 对糖尿病足和闭塞性动脉硬化症也有一定的疗效。在注射 ADSCs 的 6 个月后，从临床上看，患者的静息疼痛得到了缓解，无痛行走的距离明显延长，而且并未发现任何并发症。Vanikar 等[58]将 ADSCs 诱导成胰岛样细胞移植入 11 名 (7 名男性，4 名女性) 持续患有 1~24 年不等的糖尿病患者中，术后随访 23 个月，发现患者的外源性胰岛素用量从以前的 1.14 units/(kg BW·d) 下降到 0.63 units/(kg BW·d)，HbA1c 也从 8.47%下降到 7.39%，平均血清 C-肽从 0.1 pg/L 增加到 0.37 pg/L，患者的平均体重增加了 2.5 kg，而且患者无不适或副反应。

5 问题及前景展望

ADSCs 最大的一个优点便是含量丰富。实验表明吸脂手术吸取的每 100 mL 的脂肪组织中，ADSCs 的数量相当于等量骨髓中 BMSCs 的 40 倍，且成纤维细胞集落形成单位 (Colony-forming unit-fibroblast，CFU-F) 试验中表明，ADSCs 中干细胞数目至少是骨髓的 500 多倍[59-60]，而且 ADSCs 比其他的干细胞分化时间要短，是目前生长最快的干细胞[61]。随着肥胖症患者的增多，人类脂肪开始“富余”，并渐成为累赘。临床上脂肪抽吸术后脂肪组织大多丢弃，造成了宝贵干细胞的大量浪费。手术废弃物组织再造再利用，说明 ADSCs 又是一个廉价的干细胞来源。ADSCs 有可能成为一种廉价、可大量获得的自体干细胞，同时不存在医学伦理学和免疫排斥等问题。

目前，美国斯坦福大学的 Sun 等[62]研究人员在 ADSCs 和皮肤成纤维细胞中分别加入能够编码 4 种转录因子的基因 (*Oct4*、*Sox2*、*Klf4*、*c-MYC*) 后，约有万分之一的皮肤成纤维细胞转变为多功能干细胞 (Induced pluripotent stem cells，iPS)，而转变为 iPS 细胞的脂肪干细胞比例达到千分之二，是前者的 20 倍。利用脂肪干细胞培育的 iPS 细胞也通过了有关测试，它们能够分化成人体内的神经细胞、肌肉细胞以及肠上皮细胞等，而且所转变的 iPS 细胞安全性更高，将来有望利用 ADSCs 培育人体所需的各种器官。

但是 ADSCs 的研究甚至整个干细胞的研究目前都存在以下一些问题：1) 尚无法直接鉴定。常用的鉴定方法都是通过体外诱导多向分化，然后逆推之，才能得知是否为相应的干细胞；2) ADSCs 移植后，在体内的迁移、转归不清楚。我们以前的研究[63-64]表明，炎症期间一些细胞趋化因子具有使全身白细胞游走并迁移到创伤小鼠的创面，一氧化氮凝胶可以显著促进皮肤干细胞的形成。那么，ADSCs 移植后，是否也具有类似炎症细胞的趋向迁移性？中医中药是我国的瑰宝，是否也有相关的中药或中药制剂能够促进 ADSCs 的形成与表达？ADSCs 与中药或中药制剂对一些疾病的治疗是否具有协同作用？中药或中药制剂能否提高 ADSCs 的靶向性和有效性？3) 特异性标志物。不同的研究，不同的来源，不同的分化，ADSCs 的标志物不同，因此亟需找到 ADSCs 的特异性标志物。4) 目前国内外 ADSCs 的研究主要在体外和动物体内进行，如何将 ADSCs 的研究从实验动物走向人体，且 ADSCs 培养条件是一种优化过的环境，经过这样培养的 ADSCs 移植入人体后能否适应体内环境并保持强势增长，ADSCs 在实验动物和人体内的生长诱导环境是否相同，如果相异，相异点在哪里？这些都值得进一步地研究探讨。

总之，随着生物化学、材料化学、分子生物学、细胞生物学等的发展，ADSCs 与体细胞共培养，诱导 ADSCs 定向分化已成为确实可行有研究前景的技术，相信对 ADSCs 共培养技术及机制的深入研究，将会模拟建立更为真实的生物体微环境培养系，应用于 ADSCs 的分化诱导，为组织工程、再生医学以及各种人类疾病提供种子细胞。

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