基质血管组分(SVF)临床分离技术研究

李智国 张方方 刘悦 刘建兴 金亮*

(中国药科大学, 南京 211100)

摘要 脂肪组织易获取、组织相容性好且对供体影响小, 可作为获得成体干细胞的重要来源。基质血管组分(SVF)是从脂肪中分离出来的包括脂源性干细胞(ADSC)和基质细胞的异质性细胞群。SVF促进组织的修复和再生已被大量的临床实验所证实, 尤其是在美容整形和组织修复中的应用。早期, SVF通过酶消化法获得, 随着近年来在临床中扩大应用, 为确保患者安全和质量可控, 开发出新型自动分离设备。同时, 为符合一些国家监管要求, 避免酶的使用, 采用非酶消化法获取SVF。因此, 该文主要针对基于酶消化法和非酶消化法已经发表临床分离方法和上市的相关设备作详细论述。

关键词 基质血管组分; 酶消化法; 非酶消化法; 分离设备;

The Research of Clinical Separation of SVF (Stromal Vascular Fraction)

LI Zhiguo, ZHANG Fangfang, LIU Yue, LIU Jianxing, JIN Liang*

(*China Pharmaceutical University, Nanjing 211100, China*)

Abstract Adipose tissue is an important source of stem cells because it is easy to access and has a good histocompatibility and little influence on donors. SVF (stromal vascular fraction) is a heterogeneous group of cells isolated from adipose tissue, including ADSC (adipose derived stem cell) and stromal cells. According to a large number of clinical trials, SVF has been proved that it can promote tissue to repair and regenerate, especially in cosmetic surgery and tissue repair. In the early stage, SVF was obtained by enzyme digestion. With the extensive application of SVF in clinical practice during recent years, in order to ensure patient safety and quality control, some new automatic separation equipment was developed. Meanwhile, in order to meet the regulatory requirements of some countries and avoid the use of enzyme, non-enzymatic digestion method becomes a way of obtaining SVF. Therefore, current essay is primary to make a detailed discuss about reported clinical separation ways and related equipments based on the enzyme digestion and non-enzyme digestion.

Keywords stromal vascular fraction; enzymatic digestion; non-enzymatic digestion; separation equipment

1960年, RODBELLI[1]首次将大鼠脂肪通过胶原酶消化, 离心后脂肪分成三个不同密度层, 最下层为颗粒层, 该层包含多种细胞群体。ZUK等[2]在此方法的基础上进行改进, 通过微孔过滤从抽脂吸脂剂中分离出该细胞群, 并证明该细胞群中含有与骨髓间充质细胞具有相似表型和分化潜能的一种多能干细胞。2013年, 国际脂肪疗法与科学联合会(International Federation for Adipose Therapeutics and Science, IFAT)和国际细胞治疗学会(International Society for Cellular Therapy, ISCT)共同发表声明定义该细胞群为基质血管组分(stromal vascular fraction, SVF), 明确SVF是一种主要包括脂源性间充质干细胞(adipogenic stem cell, ADSC)、内皮/内皮祖细胞、淋巴细胞、造血系细胞、周细胞等组分的异质性细胞群[3]。SVF具有极强的血管再生和组织修复特性, 一方面归因于不同细胞具有不同的功能, 如ADSC作为一种成体干细胞具有多向分化潜能, 淋巴细胞

收稿日期: 2020-07-22 接受日期: 2020-10-23

*通讯作者。Tel: 18761874536, E-mail: ljstemcell@cpu.edu.cn

Received: July 22, 2020 Accepted: October 23, 2020

*Corresponding author. Tel: +86-18761874536, E-mail: ljstemcell@cpu.edu.cn

URL: http://www.cjcb.org/arts.asp?id=5429

诱导免疫耐受降低患者免疫反应, 内皮/祖细胞是形成血管的重要细胞群。另一方面, SVF不同细胞分泌大量的细胞因子间可相互协调作用, 如ADSC产生的血管内皮生长因子(vascular endothelial growth factor, VEGF)有助于内皮祖细胞迁移, 内皮祖细胞产生的血小板衍生生长因子(platelet derived growth factor BB, PDGF-BB)促进ADSC的增殖与迁移[4]。SVF在关节性病变、雄激素性脱发、肛瘘、血管性病变等多种疾病中均表现出良好的治疗作用, 使其具备广泛的临床应用前景[5-6]。

SVF的分离方法目前尚未统一, 基于酶的角度可被分为酶消化法和非酶消化法。酶消化法采用I型或II型胶原酶破坏脂肪细胞外基质(extracellular matrix, ECM), 非酶消化法采用乳化、离心、振荡、涡旋等机械或物理方法破坏ECM, 释放血管周围的细胞组分。酶消化法分离SVF技术成熟、细胞产量高, 缺点是成本较高、时间长、受监管和易受污染。非酶消化法分离SVF低成本、少接触和更省时, 缺点是细胞产量不高、易产生过多细胞碎屑等。针对各自的优缺点, 在酶法和非酶法的基础上, 为实现更快速、更封闭及高细胞产量等, 相继开发了一系列半自动/全自动分离提取设备, 现就SVF在酶消化法和非酶消化法基础上新开发的一些分离方法和设备进行综述, 为实验室研究和临床使用选择提供指导建议。

1 酶消化法

1.1 胶原酶

SVF分离使用的胶原酶主要是从梭状芽孢杆菌中分离纯化所得的一种中性蛋白酶, 可以有效降解ECM, 释放结缔组织中的细胞。实验研究和临床使用的酶大部分为I型和II型胶原酶, 如德国SERVA生产的NB4和NB6临床级酶, 它是I型和II型胶原酶、酪蛋白酶、梭菌蛋白酶、胰蛋白酶的混合酶。通常混合型的酶相比单一的胶原酶能分离得到更多的单核细胞, 但由于更多外源物的引入, 分离得到的细胞需进行更充分地洗涤以减少酶的残留量。

1.2 经典胶原酶法

酶消化法是获取SVF最常用也是SVF产率最高的方法。在抽脂手术中, 为获取SVF, 通常将胶原酶溶液与混合吸脂剂预先混合, 胶原酶和脂肪组织混合物经37 °C振动消化、离心、洗涤和过滤之后即可得到SVF, 分离流程见图1。利用此方法获取的SVF细胞量在10万到130万之间(每毫升脂肪), 细胞存活率在65%到93%之间。细胞得率和存活率的差异主要与酶的浓度、消化时间、吸脂部位、吸脂方式等相关。JIN等[7]采用浓度为0.1%、0.2%的I型胶原酶分别处理脂肪组织20、40、60 min, 结果显示, 用浓度为1%的胶原酶消化脂肪60 min时, 细胞产量最高。TSEKOURAS等[8]分别从人腹部、腰部、大腿内外侧抽脂并分离SVF, 大腿内侧的SVF细胞数量明显高于其他供体区域, 经过培养后, ADSC的细胞数量在93.12%~96.14%。TRIVISONNO等[9]采用传统的吸脂套管(直径3 mm, 单端口)和微套管(直径2 mm, 5个端口)抽脂, 微套管抽出脂肪颗粒更小, 微套管分离细胞数量(2.91×10^5 cells/mL)是标准套管分离SVF细胞数量的两倍(1.38×10^5 cells/mL)。

临床分离SVF为获得足够的细胞数量, 保证其再生潜能, 仍以手动分离为主。但是使用经典胶原酶法分离SVF的细胞数量和细胞活力可能存在较大的差异, 操作熟练程度和操作时间可能是造成不同批次之间治疗效果差异的原因, 并不适宜大规模处理脂肪组织。

1.3 基于胶原酶法分离设备

鉴于经典酶消化法诸多因素对SVF得率及细胞活率的影响, 以及临床使用安全性、有效性、可重复性和质量控制考虑, 相继开发了一系列半自动/自动化分离SVF设备(表1)。这些半自动/自动分离设备大多具半封闭/封闭分离环境, 分离过程与实验室手工分离流程相似, 经胶原酶处理、离心、洗涤和过滤的步骤获得SVF细胞。与手工分离不同的是, 除提供封闭环境外, 这些设备能通过调节剪切、乳化、超声等参数优化脂肪组织的前处理过程, 使脂肪颗粒的粒径更小且均一化, 降低脂肪组织粒径对SVF得率和细胞活率的影响。

根据现有临床研究报道, 临床分离使用设备以Celution®、GID-SVF1®、ADSC Extraction Kit®、Transpose RT(TPU)®、Cha-Station®等为主, 不同商业化设备在每克脂肪/吸脂剂分离的细胞产量差别100倍之多[10]。Celution®系统是报道商用系统中SVF细胞产量最高的分离系统之一, 取得欧洲统一(CONFORMITE EUROPEENNE, CE)药品和医疗器械销售许可, 可实现床旁治疗, 但目前还未经FDA批准上市, 国内尚无使用报道案列。GENTILE等[11]比

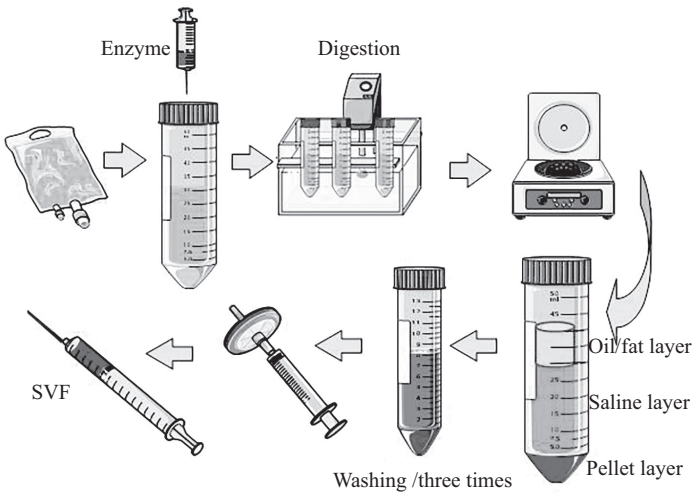


图1 经典酶法分离SVF流程

Fig.1 The process of classical enzymatic separation of SVF

较了Celution®、Medi-Khan Lipokit和Fatstem®三种分离系统, Celution®系统细胞产量(5×10^4 cells/g)最高。ARONOWITZ[10]比较了Multi Station、CHA Biotech Cha-Station、Celution 800®和Medi-Khan Lipokit四种不同分离系统, Celution 800®的分离效果最佳, 细胞产量2.4×10^5 cells/g, 细胞活力为93%。GID-SVF1®核心结构是一个完全密闭的八角筒状结构, 配套特制离心机。可大规模处理脂肪, 一次最多可处理500 mL脂肪, 并且该设备可实时检测分离的产物内毒素水平。ADSC Extraction Kit®更接近于人工分离方法, 取得的脂肪通过加入等体积酶液, 放入37 °C培养箱中慢速摇晃30 min, 最后经过离心和大量的洗涤去除酶液。Transpose RT(TPU)®系统通过模拟人工酶消化分离SVF, 包含一个自动处理室(用于组织消化、离心和洗涤)、两个注射器(用于加注胶原酶溶液和接收初成品)和细胞滤器。Cha-Station®是一个半封闭半自动的分离设备, 有一个脂肪袋、振荡离心设备、低速离心机、100 μm滤器、细胞计数器, 该设备可通过实时计数快速验证SVF的分离质量。韩国Multi Station设备是一个开放手动处理系统, 模拟人工分离过程, 设备包括一个振荡/加热器、大容量高速离心机, 所有设备均被安置在一个带有微粒空气过滤和紫外线的生物安全柜中。其他一些全封闭自动化分离设备基于产品保密协议, 未能找到相关的具体分离程序。

1.4 基于酶消化法分离的SVF的临床用途

SVF作为ADSC分离的中间产物, 相较于ADSC, 无需培养, 减少外源物接触, 同时有助于维持ADSC在体内的微态环境稳定。临床使用SVF多通过酶消化法获得, 应用于肌肉骨骼、关节、肌腱损伤、伤口愈合、泌尿生殖系统以及心血管和呼吸系统疾病领域, 并处于不同的临床研究阶段。同时为达到疗效最大化, SVF除单独使用外, 也可配合富血小板血浆(platelet-rich plasma, PRP)、纤维蛋白、常规手术或药物等其他辅助治疗手段, 部分临床试验见表2。

2 非酶消化法

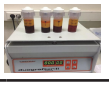
酶消化法虽然在SVF的得率上较高, 但其成本也高, 消化1 g脂肪组织所用的符合药品生产质量管理规范(Good Manufacturing Practices, GMP)级别的胶原酶成本为2美元至5美元, 同时设备分离多采用一次性耗材, 仍存在有因胶原酶洗涤不充分而导致的不良反应。此外易受监管, 酶消化法处理不符合美国食品药品安全监督管理局(Food and Drug Administration, FDA)和欧洲食品药品监督管理局(European Medicines Agency, EMA)提出的“最低实质性操作”, 认为酶消化过程可能会改变SVF的细胞生物学结构特征[37-38]。因此, 经酶消化法获取的SVF并未被FDA和EMA批准用于临床, 而非酶消化的组织样SVF(tissue SVF, tSVF)和细胞样SVF(cell SVF, cSVF)则已获批用于临床。非酶消化法主要依赖于振荡、涡旋等机械破坏力对组织进行破碎, 再通过离心或过滤等方法获取SVF。

2.1 Nanofat

Nanofat是SVF的一种, 与常规意义的细胞样SVF(cSVF)不同, 被称为组织样SVF(tSVF)。Nanofat

表1 基于酶消化法分离设备

Table 1 Separation equipments based on the enzymatic digestion

方法 Method	公司名称 Company	设备名称 Devise	细胞产量 Cell yield	细胞活力 Cell viability	图片 Picture
Semi-enclosed and automatic	Tissue Genesis, Inc	Tissue Genesis	7.02×10^5 cells/g	80.70%	
		Icellator[12-13]			
		Biosafe	…	…	
Enclosed and automatic	Cytori Therapeutics	Celution800/CRS [10-11]	5×10^4 cells/mL	63.00%	
			2.4×10^5 cells/g	93.00%	
	Stempeutics Research Pvt. Ltd	Stempeutron[14]	…	…	
	Hurim Biocell, Inc	Huricell	…	…	
	SundarRaj	Automated system[15]	1.17×10^5 cells/g	90.00%	
	Biosafe Group SA	Sepax 2[16]	2.6×10^5 cells/mL	62.00%	
Semi-enclosed and manual	Medikhan International, Inc	Lipo-Kit GT[10]	4×10^4 cells/g	72.00%	
	Eurosilicone	Puregraft 250[17]	4.25×10^5 cells/g	77.45%	
Open and manual	PNC International	MultiStation[10]	1.1×10^5 cells/g	…	
	N-Biotek, Inc	Beauty Cell	…	…	
	Cellthera, s.r.o.	Cellthera Kit I[18]	…	…	
	GeneWorld	ADSC Extraction Kit[19]	…	…	
	InGeneron, Inc	Transpose RT™ (TPU)[20]	…	88.00%	
Semi-closed semi-automatic	Cha-Station	Cha-Station[10]	1×10^4 cells/g	…	
	Medikan International, Inc	STEM-XTM	…	…	
Enclosed and manual	GID Group, Inc	GID SVF-1™[21]	7.19×10^5 cells/mL	83.00%	
	LifeCell Corporation	Revolve System[22]	…	…	
	Proteal	Stem.pras with Duografter II[17]	5.35×10^5 cells/g	69.30%	

"…"表示使用该设备分离的SVF未检测细胞产量或细胞存活率。

"…" means cell yield and cell viability of the SVF separated by the equipment are not tested.

表2 基于酶消化法临床分离方法

Table 2 Clinical separation method based on the enzymatic digestion

临床用途 Clinical application	作者 Author	分离方法 Separation method	给药方式 Way of administration	临床阶段 Level of clinical	患者数量 Number of patient
Bone/Joint/Tendon injuries	BANSAL[23]	Open and manual	SVF+PRP	Case series level IV	*n*=10
	HONG[24]	Open and manual	SVF	Level II	*n*=16
	KIM/CHOI[25]	Open and manual	SVF+fibrous protein	Case series level IV	*n*=17
	KOH/KWON/KI[26]	Open and manual	SVF+high level osteotomy +PRP	Level II	*n*=21
	PAK[27]	Open and manual	cSVF+PRP+HA	Case series level IV	*n*=3
Chronic wounds	CARSTEN[28]	GID SVF-1,	SVF	Case series level IV	*n*=10
	CERVELLI[29]	Celution®	SVF+PRP	Level III	*n*=10
	HAN[30]	Open and manual	SVF+fibrous protein	Level II	*n*=26
Urogenital injury	MIZUSHIMA[31]	Celution system	SVF+fibrous protein glue	Case series level IV	*n*=6
	ANDELKOV[32]	Celution®800	SVF	Case series level IV	*n*=6
Cardiovascul/ pulmonary diseases	COMELLA[33]	Adipolase™	SVF	Case series level IV	*n*=28
Peripheral nerve injury	MAUSKOP[34]	Open and manual	SVF	Case series level IV	*n*=9
	BRIGHT[35]	Open and manual	cSVF+PRFG	Case series level IV	*n*=4
	CALGAGNI[36]	Celution®800	SVF+fat transplantation	Case series level IV	*n*=5

通过乳化作用破坏成熟脂肪细胞, 获得一种含有cSVF和细胞因子的凝结物, 在微整形领域具有较广泛应用。Nanofat制备通过使用直径为1 mm的锋利侧孔的多端口3 mm套管收集微型脂肪, 之后在两个10 mL的注射器中互推30次, 直至脂肪变白成为液体状, 之后用无菌纱布过滤, 滤液被称为Nanofat[39-40]。这种Nanofat能够直接注射和制备皮肤贴片使皮肤自主吸收[41], 适用于面部皮肤老化修复和胸部重塑。Nanofat能充分保持ADSC的干性, 适用于提高脂肪移植物保留时间, 但乳化过程产生过多的细胞碎屑和油脂, 会引发局部的炎症反应, cSVF相较于Nanofat, 细胞富集程度更高, 避免炎症反应且更适用于局部病灶的微量注射。

2.2 基于非酶手动分离SVF

人工手动分离SVF是实验室常用的一种非酶分离方法, 采用离心、振荡、摇晃等方法破坏ECM释放基质细胞, 但是标准分离程序存在较大争议, 分离的细胞量因人而异, 一些手动分离方法见表3和图2。离心一般采用梯度离心法, 速度控制在200 ×*g*~500 ×*g*, 离心速度需要控制在保持基质细胞的完整性, 同时破坏脂肪细胞的范围内。由于脂肪细胞破坏后会释放大量油脂, 需要充分离心洗涤, 避免残留过多油脂而引起机体发生炎症反应。机械振荡指使用高速机械振荡的方法打破ECM对细胞的束缚。如RAPOSIO等[42]采用6 000次/min的高速振荡仪持续振荡6 min, 脂源性干细胞约占的总细胞数的5%。CHAPUT等[43]采用3 200次/min涡流6 min, 最后涡流法细胞存活率仅为酶解法的一半, 但是细胞集落数量(colony forming unit, CFU)明显高于酶解组。

2.3 基于非酶分离设备

在实验室手动分离的基础上, SVF的分离技术有所改进, 一些自动分离设备相继被开发(表4)。本文把一些套装全手工分离设备也归类为半自动分离设备, 市场已经有一些设备/套组获得上市许可, 但是分离的SVF细胞数量、存活率、各类细胞比例程度有较大差异, 操作者熟练水平、脂肪质量、操作环境是造成差异的主要原因。

TIRYAKI等[52]采用自制封闭立方体装置分离SVF, 该设备结合了机械分离、缓冲培养和离心步骤。该设备含有一个三通立方体腔室, 立方体三面分别装载1 000 μm、750 μm和500 μm的金属滤膜, 该立方体的三面出液口均为鲁尔口, 可同时连接三个注射器, 滤膜从大到小顺序依次连续推动相应的注射器, 脂肪呈现乳糜状后离心取下层, 即为SVF层。通过对35例妇女脂肪进行自制设备机械消化处理和酶消化处理脂肪进行对比, 结果发现, 酶消化细胞产量(3.38×10^6 cells/mL)高于机械消化(1.34×10^6 cells/mL), 该自制设备保证了乳化操作的密封性, 同时也是唯

表3 基于非酶手动分离方法

Table 3 Manual separation methods based on the non-enzymatic digestion

方法 Method	作者 Author	细胞产量/脂肪 Cell yield/adipose tissue	细胞活力 Cell viability	ADSC细胞量/脂肪 ADSC yield/adipose tissue
High speed centrifugation	GONTIJO-DE-AMORIM[44]	…	…	1.62×10^3 cells/mL
	MARKARIAN[45]	2.5×10^4 cells/mL	65.00%	…
	BAPTISTA[46]	2.40×10^5 cells/mL	…	1.200×10^4 cells/mL
	CONDE-GREEN[47]	2.30×10^4 cells/mL	…	…
High speed shock	ROMANOV[48]	…	…	…
	RAPOSIO[49]	1.56×10^3 cells/mL	…	6.25×10^3 cells/mL
	CHAPUT[43]	…	54.53%	1.00×10^5 cells/g
Emulsification	CHAPUT[43]	…	45.53%	5.00×10^4 cells/g
Vortex	CONDE-GREEN[47]	1.20×10^4 cells/mL	80%-90%	1.56×10^3 cells/mL
Shake violently	SHAN[50]	…	…	2.50×10^4 cells/mL
Ultrasonic cavitation	BRIGHT[51]	…	…	…

“…”表示使用该设备分离的SVF未检测细胞产量或细胞活力。

“…” means cell yield and cell viability of the SVF separated by the equipment are not tested.

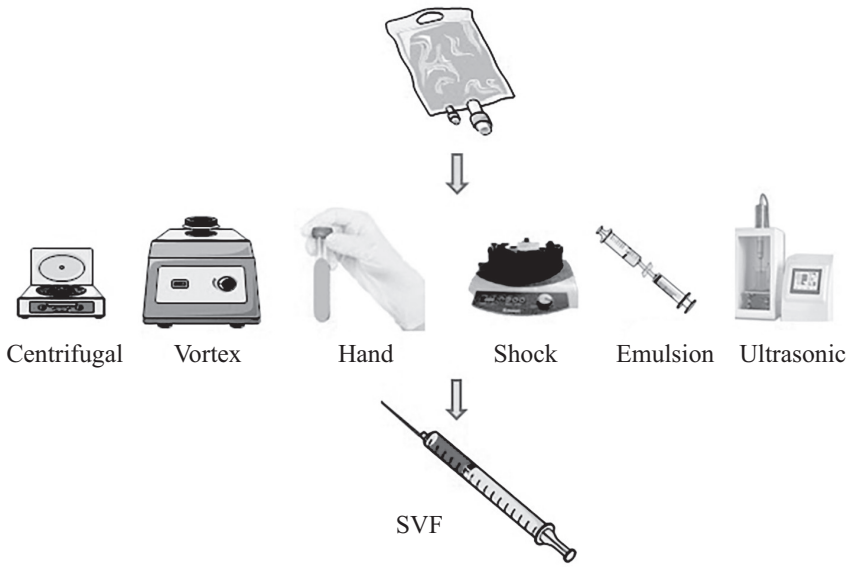


图2 基于非酶消化手动分离SVF方法(根据参考文献[57]修改)

Fig.2 Manual separation of SVF based on non-enzymatic digestion (modified from reference [57])

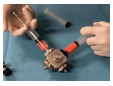
一已被证明非酶法分离的细胞产量比酶法高的设备。德国Arthrex ACA kit[53]套组设备原本用途是分离PRP, 也有被用来分离SVF的报道, 该设备采用的核心部件为双针筒可拆卸注射器。采用30 mL的吸脂剂经过第一次低速离心后, 取中层脂肪, 通过鲁尔端连接两个注射器, 两个注射器来回连续推动乳化60次, 进行第二次离心, 底层即为SVF细胞层。该套组需要配置特殊的离心机, 价格相比其他设备较昂贵。意大利Lipogems产品[54-55]在2011年获得国际专利, 最初被用于脂肪移植过程中的富集脂肪步骤, 后期经过改良发展成为提取tSVF细胞的产品之一。采用一个完全封闭的系统、无酶、少剪切力分离SVF。核心部件为一根规格为250 mL的圆柱容器, 圆柱两端各有两个孔径不同的滤膜(灰色滤膜和蓝色滤膜), 三通管可接注射器和溶液袋。Fatstem kit套组采用过滤、离心步骤直接分离SVF。先将获得的吸脂剂通过含有滤网的输液袋, 过滤除去大颗粒脂肪块, 收集的脂肪以1 700 r/min转速离心, 最下层为SVF细胞层, 获得细胞悬液通过100 μm的滤器得到SVF。myStem®设备是一个密封的设备, 上端直接注入脂肪, 下端即可收获SVF, 细胞产量最低。

2.4 基于非酶消化法临床分离方法

基于非酶消化, 手动和套组分离所获SVF均有应用于临床的报道。截止2020年, 基于非酶消化法开展

表4 基于非酶消化分离设备

Table 4 Separation equipments based on the non-enzymatic digestion

公司/实施人 Company/reference	设备名称 Devise	细胞产量 Cell yield	细胞活力 Cell viability	图片 Picture
Tiryaki[52]	Closed cube	1.34×10^6 cells/mL	85.82%	
Arthrex[53]	Arthrex ACA kit®	…	…	
Rigenera[56]	Rigenera®	…	…	
Lipogems International[54-56]	Lipogems®	…	…	
CORIOS Soc. Coop[11]	Fatstem kit	3×10^4 cells/mL	52.00%	
MyStem LLC[11-57]	myStem®	8×10^3 cells/mL	43.00%	

“…”表示使用该设备分离的SVF未检测细胞产量或细胞存活率。

“…” means cell yield and cell viability of the SVF separated by the equipment are not tested.

表5 基于非酶消化法临床分离方法

Table 5 Clinical separation method based on the non-enzymatic digestion

临床应用 Clinical application	作者 Author	分离方法 Separation method	给药方式 Way of administration	临床阶段 Level of clinical	患者数量 Number of patient
Skin regeneration	CHARLES-DE-SÁ[58]	Centrifugation	Hypodermic injection	Case series level IV	*n*=6
	COHEN[59]	Emulsification	Skin surface puncture	Case series level IV	*n*=50
Wound healing	TONNARD[39]	Emulsification	Hypodermic injection	Case series level IV	*n*=67
	TARALLO[60]	MyStem®	Multipoint iniection	Case series level IV	*n*=40
Amputation wound recovery	LONARDI[61]	Lipogems®	Multipoint iniection	Case series level IV	*n*=114
Tendon injury	ALBANO[62]	FastKit®	Intra-endon injection	Case series level IV	*n*=21

SVF的临床研究试验中, 全球统计有35项关于SVF临床研究报告, 涉及到1 405名临床试验对象。总体上基于SVF均有良好治疗效果, 有50例轻微不良反应和1例严重不良反应[5]。其中使用的方法主要是离心法、乳化制备Nanofat、商用设备等, 部分临床试验见表5。

3 总结与展望

随着SVF分离技术的发展, SVF细胞治疗在越来越多的临床病症中被证实效果良好, 显示出广阔的应用前景。酶消化法仍然是SVF的主流方法, 其方法成熟、细胞产量和存活率较高, 正朝着封闭自动化水平发展。但是目前对于酶的使用, 各国的法律意见不一, 达成的统一共识是尽量避免引入外源性物质。因此为规避监管, 一是基于酶消化法的SVF分离设备通常作为一种医疗用品而不作为医疗器械申请上市。二是无酶机械分离可作为一个替代方法, 但机械分离SVF细胞产量低、方法不成熟、缺乏商用自动化设备, 所以机械分离正处于起步阶段。临床上分离SVF, 无论是基于酶消化法和非酶法, 均需要减少外源交叉污染、操作人员依赖性、提高可重复性和简化分离技术, 虽然目前已经有全自动封闭酶消化设备, 但是目前尚缺少一些比较性的研究, 这些结果的发表, 将有利于制定出符合临床级SVF分离操作标准。

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