

# 干细胞及其仿生基质微环境在关节软骨再生修复中的作用综述\*

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**【摘要】** 关节软骨由于无神经、无血管的特性,一旦损伤,其自我修复十分困难。干细胞技术的发展为关节软骨再生提供了新的希望。当前,不同来源的干细胞及多种应用方式在关节软骨修复中展现出不同程度的治疗效果。然而,干细胞对其所处的微环境均有极强的敏感性,这使得越来越多的研究者开始关注通过生物功能性支架构建的仿生微环境来调控干细胞,进而加速软骨再生。本文主要讨论了软骨修复的干细胞来源、其应用手段及其协同仿生细胞微环境在关节软骨修复方面的治疗效果、机制和应用不足。希望对协同干细胞的功能性软骨修复支架的设计和 optimization 提供更加切实的临床化研究思路。

**【关键词】** 关节软骨修复 干细胞 生物功能性支架材料 仿生细胞微环境

**Role of Stem Cells and Their Biomimetic Matrix Microenvironment in Regenerative Repair of Articular Cartilage: A Review** CAO Hong-fu<sup>1,2</sup>, LI Zhu-lian<sup>1,2</sup>, SUN Yong<sup>1,2△</sup>, FAN Yu-jiang<sup>1,2</sup>, ZHANG Xing-dong<sup>1,2</sup>. 1. National Engineering Research Center for Biomedical Materials, Sichuan University, Chengdu 610064, China; 2. College of Biomedical Engineering, Sichuan University, Chengdu 610064, China

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**【Abstract】** It is difficult for the articular cartilage to self-heal any damage it may incur due to its lack of nerves and blood vessels. Development in stem cell technology provides new prospects for articular cartilage regeneration. Currently, stem cells from different sources and their diverse applications have demonstrated different degrees of therapeutic effect and potential in articular cartilage repair. However, stem cells are highly sensitive to their microenvironment. Therefore, more and more researchers are focusing their attention on regulating stem cells and thus accelerating cartilage regeneration through the biomimetic microenvironment constructed by biologically functional scaffolds. We reviewed in this paper the sources of the stem cells used for cartilage repair, the application method of these stem cells, as well as the therapeutic effect, mechanism and limitations in the application of stem cells synergizing with the biomimetic microenvironment in promoting articular cartilage repair and regeneration. We hoped to provide suggestions for practical clinical research in the design and improvement of biofunctional cartilage repair scaffolds that synergize with stem cells.

**【Key words】** Articular cartilage repair Stem cell Biofunctional scaffold materials Biomimetic microenvironment

关节软骨覆盖并保护着关节处的长骨末端,具有负重和润滑减少摩擦的能力<sup>[1]</sup>。由于其无血管和无神经的特性,一旦损伤,其修复和再生非常困难,这已经成为再生医学领域最具挑战性的研究热点之一<sup>[2]</sup>。临床上,保守治疗(如关节腔注射药物、中医疗法等)<sup>[3]</sup>和手术治疗(清创术、细胞移植术、微骨折术以及软骨移植术等)<sup>[4-5]</sup>均有一定的局限性,只能部分缓解疼痛、改善关节功能,未能有效地实现关节软骨结构和生物功能的重建。组织工程和再生医学的发展为骨关节软骨的缺损修复提供了新的期待(图1)。软骨细胞作为关节软骨内的主要驻留细胞,

是软骨组织工程中最早使用的细胞类型,但自体软骨细胞的使用需要二次手术,易增加供区的软骨损伤,且新生部分和原位组织的整合欠佳<sup>[6-7]</sup>。干细胞在软骨发育的早期阶段凝集和分化<sup>[8]</sup>,它具有多向分化潜能,能在特定诱导条件下分化成软骨细胞,被认为是软骨再生修复中的自体细胞治疗和再生策略的最佳来源<sup>[9-10]</sup>。研究表明,干细胞在其局部微环境中感知细胞外基质(extracellular matrix, ECM)组成变化的能力对它们的生存和命运至关重要<sup>[11]</sup>。

因此,由天然和合成聚合物形成的生物功能性支架可模仿天然细胞外基质(ECM)的许多生物学和物理特性<sup>[12-13]</sup>,调控干细胞的增殖和定向分化潜能<sup>[14]</sup>。这一独特机制为关节软骨的修复与再生提供了新的有效途径。本文将围绕关节软骨修复,从干细胞来源,协同干细胞的治

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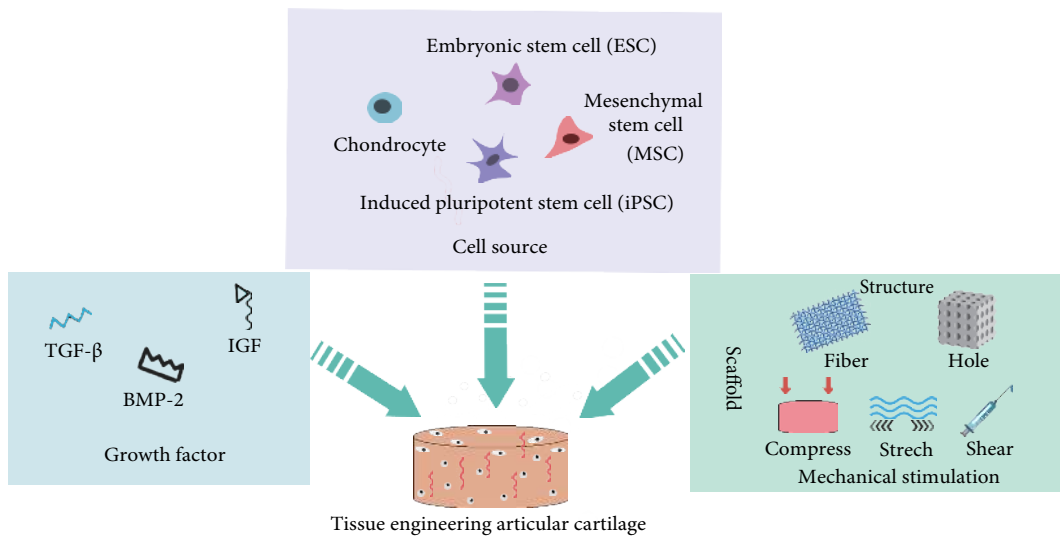


图 1 组织工程关节软骨三要素

Fig 1 Three elements of tissue engineering articular cartilage

TGF-β: Transforming growth factor-β; BMP-2: Bone morphogenetic protein-2; IGF: Insulin-like growth factor.

疗方式, 以及干细胞与仿生基质微环境的交互作用三个方面综述其作用机制、修复效果和临床转化难点。

## 1 干细胞在关节软骨修复中的应用

### 1.1 干细胞种类和来源

间充质干细胞(mesenchymal stem cell, MSC)、胚胎干细胞(embryonic stem cell, ESC)和诱导多功能干细胞

(induced pluripotent stem cell, iPSC)是干细胞治疗的主要候选细胞系<sup>[15]</sup>。

**1.1.1 MSC** MSC是一类具有多种生长潜能、形态和功能独特的细胞, 它们具有的软骨形成潜能和强力的免疫抑制能力等有助于关节软骨的修复<sup>[16-17]</sup>。近来, 各种MSC均已用于软骨细胞再生, 呈现出不同程度的修复潜能(表1)。

表 1 不同MSC的成软骨分化的优缺点

Table 1 Strengths and weaknesses of chondrogenic differentiation of different mesenchymal stem cells

| Cell source  | Advantage   | Disadvantage   | References |
|--|---|--|------------|
| Bone marrow mesenchymal stem cell (BMSC)                     | One of the most common and effective sources for the treatment of cartilage damage.   | Clinically, it is relatively difficult to extract BMSCs, and the sampling process is highly invasive, while the amount is limited. | [18-20]    |
| Adipose derived mesenchymal stem cell (ADSC)                 | ADSC is widely derived and easy to obtain, can secrete a variety of cytokines, and has high proliferative capacity and multidirectional differentiation potential.              | The chondrogenic potential is lower than that of BMSCs.  | [21-22]    |
| Human umbilical cord derived mesenchymal stem cell (hUC-MSC) | hUC-MSC is easy to isolate and culture, and has less ethical issues, painless harvest process, high cell proliferation, broad differentiation potential and low immunogenicity. | Most of them are allografts.   | [23-24]    |
| Umbilical cord blood mesenchymal stem cell (UCB-MSC)         | Noninvasive collection, showing very high proliferative capacity.   | Most of them are allografts.   | [25-26]    |
| Synovial mesenchymal stem cell (SMSC)                        | SMSC has greater chondrogenic potential than MSC from other sources.  | SMSC is difficult to extract and there is only limited research.   | [27]       |

**1.1.2 ESC** ESC源自哺乳动物胚泡的内部细胞团, 能够无限增殖, 并可以分化成不同的终末细胞。细胞外基质的组成、表面粗糙度以及几何构型都对ESC在向软骨细胞分化过程中有很大的影响。多项研究表明, 细胞因子或与其他细胞共培养等诱导方式可有效提高ESC的体外软骨分化特性<sup>[28]</sup>。但遗憾的是ESC的临床研究及应用仍受到其伦理问题以及致癌性的阻碍。

**1.1.3 iPSC** iPSC为软骨组织工程技术提供了新的种子

细胞来源。iPSC可以通过逆转录病毒介导的4个转录因子, 即*Oct3/4*、*Sox2*、*c-Myc*和*Klf4*的转染, 从小鼠胚胎成纤维细胞和成年小鼠尾尖成纤维细胞中产生<sup>[29]</sup>。iPSC与ESC在细胞形态、生长特性、基因表达等方面具有相似性, 此外还有来源广泛和个体疾病特异性等优势。研究表明把iPSC直接植入具有免疫缺陷的模型小鼠体内将会造成畸胎瘤<sup>[30-31]</sup>, 因此在将iPSC应用于软骨修复再生治疗前, 要先通过诱导分化, 使其分化为软骨细胞。目前, 使

iPSC分化为软骨细胞的策略主要有3类:与原代软骨细胞共培养、拟胚体途径的软骨细胞分化、MSC途径的软骨细胞分化<sup>[32]</sup>。iPSC虽能够弥补自体软骨细胞的缺点,但

高成本和低效率限制了它们的应用。

### 1.2 干细胞的无支架应用方式和效果

干细胞常见的3种无支架应用方式如图2所示。

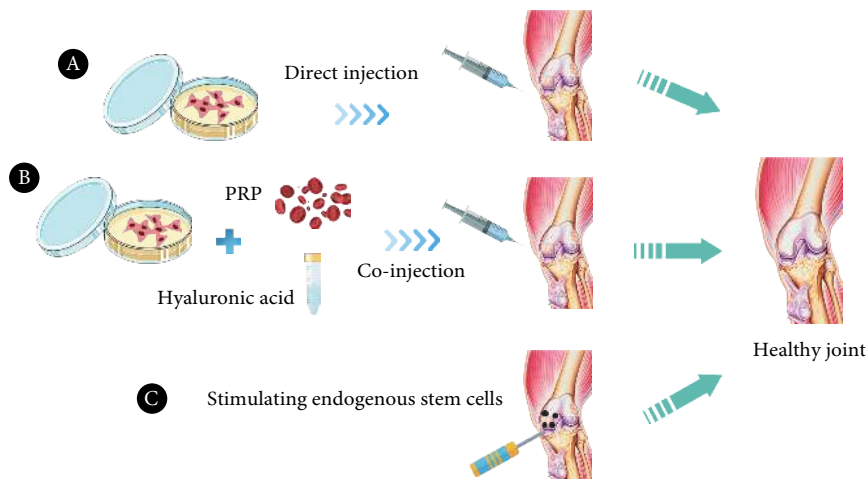


图2 无支架干细胞应用于关节软骨修复的方式

Fig 2 Application of scaffold-free stem cells in articular cartilage repair

A: Direct intraarticular injection of stem cells; B: Co-injection of auxiliary active ingredients; C: Microfracture, utilizing endogenous stem cells; PRP: Platelet rich plasma.

**1.2.1 直接注射** 当前,用于治疗骨关节炎及其造成的关节软骨损伤最常见的方法是无支架方法,即以生理盐水作为载体,向关节腔注射干细胞。研究表明,关节内注射的干细胞多为自体MSC,能有效促进软骨缺损再生,且在一定浓度范围内,修复效果与细胞浓度呈正相关<sup>[33-34]</sup>。临床上通过膝关节损伤、骨关节炎评分,结合膝关节磁共振成像(MRI)和关节镜检查,证明关节腔内注射MSC对膝关节关节炎患者的功能改善、症状缓解甚至软骨再生都有一定的治疗效果<sup>[35]</sup>。同时也有研究表明,膝关节多次注射MSC的临床效果更好<sup>[36]</sup>。

**1.2.2 联合其他有效成分** 除了直接注射MSC,也可将富血小板血浆(platelet rich plasma, PRP)和关节润滑剂透明质酸(hyaluronic acid, HA)作为载体,联合MSC进行关节腔内注射。研究表明,联合注射法使得MSC的增殖和软骨分化能力均有提升,通过MRI等手段检测也进一步证实了最佳的软骨再生情况。PRP含有丰富的生长因子,可促进MSC向软骨分化<sup>[37]</sup>,部分HA在软骨和滑膜表面聚集,一方面重新恢复已破坏的生理屏障,防止软骨基质的进一步丢失,一方面附着在关节软骨表面,降低被代谢的速率,为MSC提供保护。

**1.2.3 刺激内源性干细胞自我修复** 关节软骨下骨的骨髓腔内有丰富的骨髓间充质干细胞(BMSC),通过微骨折技术可使软骨下骨出血释放干细胞及生长因子,进入软骨面促进软骨再生,其再生能力远高于软骨细胞迁移进行的修复<sup>[38]</sup>。但BMSC和生长因子难以保留在缺损部位

而是多被释放到关节中,缺损部位不能受到持续有效调控,最终纤维化严重。因此,微骨折术短期术后效果好,但纤维组织的机械质量差,不能达到正常的透明软骨的承重及运动效果,术后长期效果仍不理想<sup>[39]</sup>。

### 1.3 干细胞与支架材料构建仿生基质微环境交互作用再生软骨缺损

研究发现MSC促进关节软骨修复的机制有两方面:自身分化形成软骨细胞和通过旁分泌作用释放活性因子促进周边细胞向缺损组织转化。体外实验表明,在多种生长因子(如转化生长因子- $\beta$ <sup>[40]</sup>)、药物(如地塞米松)及环境(如低氧、机械信号<sup>[41-42]</sup>)的作用下,可促进MSC的成软骨分化。近来,越来越多的研究认为旁分泌作用可能在关节软骨修复中发挥最大作用<sup>[43-44]</sup>。MSC在适当微环境下会分泌相应的细胞活性因子,促进关节软骨周围细胞再生。另外,徐立璋等<sup>[45]</sup>发现BMSC旁分泌对于软骨细胞IL-1 $\beta$ 损伤后有保护作用。综上,生物功能性支架材料展现了极大的潜力,可以对细胞起到保护作用,同时通过生物材料复合干细胞构建不同的仿生基质微环境调控干细胞的分化及旁分泌作用。

## 2 干细胞协同生物功能性支架修复软骨缺损的机制

### 2.1 调控外源性干细胞再生软骨缺损

**2.1.1 促进增殖和分化** 软骨诱导性生物材料的研究越来越多,多种天然或合成的高分子材料均被报道有促进

干细胞成软骨分化的潜力。天然材料由于其良好的生物相容性和生物降解性被广泛应用,如I型胶原、明胶、丝素蛋白和软骨脱细胞基质等,均被报道有良好的软骨诱导性<sup>[46-49]</sup>。这些天然材料提供了类似于天然软骨的微环境,可以促进干细胞的黏附、向软骨分化及软骨基质的产生。

研究发现人工合成的高分子聚合物一般有较好的力学性能,可以给细胞提供良好的保护作用。另外,通过改变合成参数可以赋予生物功能性支架材料不同的特性以制造不同的细胞微环境。如于庆贺<sup>[50]</sup>制备出了可调整刚度的聚丙烯酰胺凝胶,发现刚度较低的细胞外基质上的人类脂肪干细胞(human adipose-derived stem cells, hASCs)增殖更慢,但成软骨分化水平更高。洪雅真等<sup>[51]</sup>研究发现纳米纤维结构支架能够促进细胞成软骨分化。

综上,结合天然材料和合成材料的优势,优化组成结构、生物力学性能和生物功能性,构建仿生基质微环境调控干细胞定向分化和增殖,有利于加速软骨再生。

**2.1.2 促进旁分泌** 使用生物功能性支架对干细胞旁分泌进行辅助,一方面可以指导干细胞进行特定的细胞因子的分泌,另一方面还可以有效递送细胞分泌的生长因子等信号。根据报道,不同生物功能性的支架材料在调节MSC增殖和分化中起重要作用(在2.1.1中已经部分列举)。有研究表明基质刚度(弹性)会影响旁分泌活性,当将MSC培养在不同刚度的聚丙烯酰胺水凝胶上时,VEGF和IGF随模量增加而上调,而EGF、IL-6和IL-8显示出双相分泌特征<sup>[52]</sup>。这表明,当需要特定的旁分泌因子时,可以通过优化MSC微环境的机械性能或者其它性能对干细胞的旁分泌进行调控。

有些生物功能性支架材料在递送细胞因子方面有独特优势。例如HA,它的结构和生物学特性介导其在细胞信号传导方面有重要作用。HA能与许多干细胞表面受体强烈相互作用,进而影响细胞的活性、增殖速率、迁移情况和分化趋势,主要受体之一是CD44。此外,与基础细胞骨架的密切联系使CD44可以启动细胞内信号传导,从而使其能够感知ECM环境中的变化并发出细胞应答信号<sup>[53-54]</sup>。

另外,生物功能性支架的孔隙对于细胞旁分泌的作用效果也有影响。QAZI等<sup>[55]</sup>通过将细胞与离子交联的RGD修饰的藻酸盐结合在一起,可以生产出两种类型的水凝胶-MSC构建体,即纳米多孔水凝胶(10 nm)和微米多孔水凝胶(125 μm),研究发现可溶性生长因子能在微孔而非纳米孔水凝胶中放大MSC旁分泌活性。这也进一步提示我们,在材料制备及选择时应考虑材料结构及特

性对细胞旁分泌因子递送的影响。

**2.1.3 调节免疫应答** 骨关节炎、类风湿性关节炎常常引起软骨损伤,这种软骨损伤常伴随着复杂的炎症反应。一些生物功能性支架材料已经被报道能辅助干细胞调节炎症反应。不同分子量的HA会在细胞行为上引发不同的炎症反应。低分子量HA(LMWHA)产生并维持炎症反应,并通过巨噬细胞和树突状细胞上的TLR-2信号传导;高分子量HA(HMWHA)不是促炎性介质,其能抑制TLR-2信号传导<sup>[56]</sup>。外源性HA可能通过调节多糖蛋白的表达以及NF-κB信号通路分子的表达来调节炎症反应<sup>[57]</sup>。有报道表明羧甲基壳聚糖可能通过抑制骨关节炎大鼠软骨细胞I-κB的降解,从而抑制NF-κB信号通路的活化,进而下调骨关节炎大鼠软骨iNOS mRNA和蛋白的表达,对骨关节软骨起到保护作用<sup>[58]</sup>。可见,具有免疫应答调节的生物材料对于软骨修复具有潜力。

## 2.2 调控内源性干细胞再生关节软骨

仿生基质微环境调控内源性干细胞再生关节软骨已经逐渐成为研究热点。基质诱导软骨再生技术(matrix induced cartilage regeneration, AMIC)是无细胞支架结合微骨折术共同完成软骨修复的手段,能一定程度改善微骨折技术的局限性,如能更好的固定内源性骨髓干细胞和生长因子,并提供更好的机械应力<sup>[59-60]</sup>,使MSC向内生长,从而再生软骨。已经有I型胶原软骨基质(Ca Re S<sup>®</sup>-1S, 关节动力,德国)被应用于基质诱导软骨再生技术,在临床应用重展现出良好的安全性并取得了良好的近期疗效<sup>[61]</sup>。

为了更好地发挥作用,此种无细胞支架材料还应具有一定独特的功能,如细胞招募能力、促进细胞迁移或促进细胞黏附等。有研究表明ECM的物理性能可以影响细胞迁移,例如强度、交联方式和孔径<sup>[62]</sup>。还有研究表明,尽管细胞在凸表面上的分布比在凹表面上的分布更多,但凹面的移动方向的突出力大小比凸面大,促进了细胞在凹面上的持久迁移。例如包裹在微米级凸底物(例如胶原束)上的细胞移动效率会很低。但是,如果胶原蛋白束对齐,则细胞迁移模式可能会切换为更持久的迁移,因为对齐的束之间的细胞实际上将在凹形底物上移动<sup>[63]</sup>。因此,无细胞支架材料的设计还要综合考虑多个方面,如细胞黏附、细胞迁移、细胞固定以及良好生物相容性等。

## 3 存在的问题及展望

目前修复及再生受损的软骨仍是临床面临的挑战,尽管各种手术和非手术方式取得了一定的疗效,但是都存在一定的缺陷。而运用组织工程学修复关节软骨虽取得了一定进展,但现有的各种生物功能性支架材料的研

制与开发仍未能完全满足再生软骨的需求。本文对不同仿生基质微环境促进不同来源的干细胞再生关节软骨进行综述,对材料设计提出了几个有针对性的思考方向。

利用干细胞进行关节软骨缺损治疗的研究越来越多,目前临床上多采用自体MSC进行相关研究,可以降低免疫排斥反应。但自体干细胞如BMSC的提取侵袭性强,且为达到使用数量要求仍需体外进一步扩增。因此调控内源性干细胞促进软骨修复的无细胞的生物功能性支架具有良好的临床应用前景,但内源性干细胞数量受限,达到稳定有效的修复效果难度较大,相应的生物功能性支架的优化设计仍有待进一步提升。

此外,干细胞在临床应用中出现的問題已经得到了广泛关注,例如畸胎瘤、免疫排斥反应,批次和剂量依赖性作用之间的非稳定性。干细胞衍生物产品(例如外泌体等)的开发则显得十分必要。相比于干细胞的直接使用,衍生物有较大的优势:无免疫排斥,体积小穿透力强,可避免伦理道德争议,加速临床审批。因此,利用干细胞衍生物协同功能性生物材料构筑再生修复微环境可能是软骨损伤或骨关节炎干细胞疗法的有前途的替代方法之一,这可能提升“无细胞”疗法的临床应用潜力。

\* \* \*

**利益冲突** 所有作者均声明不存在利益冲突

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