**富血小板血浆及血小板来源的细胞外囊泡在治疗膝关节骨性关节炎中的应用**

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**摘要：**膝关节骨性关节炎(Knee osteoarthritis, KOA)多发于老年人、主要引起膝关节退行性改变，出现关节疼痛、肿胀、僵硬等症状，是一种严重影响患者生活质量和身心健康的临床常见疾病。目前膝关节骨性关节炎治疗包括保守治疗方法和手术治疗，随着医疗技术的不断发展，非手术治疗膝关节骨性关节炎取得了明显的疗效。富血小板血浆(Platelet-rich plasma，PRP)以及血小板外囊泡目前已在临床多个学科应用，全面了解PRP以及PEVs参与KOA的机制，为治疗KOA提供夯实的理论支持。本文拟从PRP以及PEVs治疗KOA的应用进行综述，为临床治疗KOA提供理论支持。

**关键词：**富血小板血浆；血小板来源的细胞外囊泡；膝关节骨性关节炎

**前言**

骨性关节炎（Osteoarthritis，OA）是最常见的非对称性、无菌性和无全身性征象的退行性关节疾病，好发于老年人以及发病率随年龄增长而增加，影响全球2.4亿人的健康，男/女性分别占10%、18%，严重影响患者的生活质量和劳动能力，被WHO称为“不死癌症”[1, 2]。膝关节是一个复杂的器官，包括关节软骨、滑膜、髌内脂肪垫、半月板、关节周围肌肉、韧带和肌腱等多个结构互相影响[3, 4]。膝关节骨性关节炎（knee osteoarthritis，KOA）是OA类型中最常见的病症，临床表现为进行性的膝关节疼痛、肿胀、僵硬以及活动受限，是由于软骨基质的合成与分解代谢失衡，从而导致的关节软骨破坏[5, 6]；特别是，因外伤或高强度的工作出现滑膜炎、半月板损伤、软骨损伤等自行服药没有及时就诊，延误了最佳的诊治时间使病程延长[7-9]。目前临床治疗KOA的主要手段包括保守治疗和手术治疗，保守治疗包括运动治疗、矫形治疗、使用镇痛药或非甾体抗炎药物等[10-12]。目前，全膝关节置换术治疗KOA效果显著，但手术费用较高、出院后患者跌倒的风险大[13]、甚至后期人工关节可能需要翻修[14]等导致KOA手术治疗具有一定的局限性。因此，开发新的、安全高效的方案对治疗KOA来说至关重要。近年来，为满足针对治疗KOA的需要，临床上使用富血小板血浆（Platelet-rich plasma，PRP）[15, 16]以及血小板来源的细胞外囊泡[17]（extracellular vesicles, PEVs）治疗KOA取得了一定的治疗效果。

富血小板血浆（Platelet-rich plasma，PRP）含有丰富的血小板，其数目约为全血的3倍，血小板在维持体内平衡和组织修复[18]中起关键作用。血小板被激活后会释放大量的生长因子促进各种组织修复与再生、参与调节免疫系统，特别是多种炎症过程，影响正常的白细胞生物学功能和炎症信号[19]。有文献表明膝关节腔内注射纯富血小板血浆（Pure platelet-rich plasma；P-PRP）或者生理盐水6个月后，注射P-PRP的患者关节液中TNF-α和IL-1β水平较低。术后60个月，注射P-PRP的胫股关节软骨体积平均减少1171 mm3，仅注射生理盐水的患者胫股关节软骨体积平均减少2311 mm3[20]；PRP联合透明质酸治疗KOA显著降低VAS，这种治疗方法有助于减轻轻/中度KOA患者的疼痛，且在改善膝关节活动能力和功能方面效果更好[21]。PRP在临床上针对骨性疾病具有较好的治疗效果；且不同种类的PRP治疗效果不尽相同。

细胞外囊泡（extracellular vesicles, EVs）是一种由细胞分泌到细胞外的双膜闭合囊泡，包括脱落微囊泡(microvesicles，MVs；50 nm-1000 nm in diameter)、外泌体(exosomes，Exos；30-150 nm in diameter)和凋亡小体(apoptotic bodies；100 nm-5000 nm in diamete)三种类型[22-24]；EVs携带脂质、蛋白质和RNA等多种具有生物学功能活性分子，广泛参与细胞间信号传递，在作为治疗性分子载体方面具有巨大潜力[25-27]。有研究表明，星形胶质细胞来源的EVs能够促进脑缺血后血管新生，对脑缺血后损伤的神经元具有保护作用[28]；Faruk等学者认为间充质干细胞来源EVs促进细胞的修复能力[29]。此外，血小板被激活后分泌颗粒内容物从细胞表面脱落并释放PEVs，可能影响凝血、免疫反应、炎症、血管生成、伤口愈合、组织修复和癌变等各种重要的生理和病理功能[30-32]。Tao等报道PEVs参与调节信号转导，是PRP的高效替代品，也是药物或其他分子（mRNA、miRNA和lncRNA）给药的潜在载体[33]；这些研究显示，在调节许多疾病的细胞活性时，PEVs是一种较优的选择。

**PRP、PEVs的制备及分类**

根据血液成分不同进行细胞密度离心，从浓缩装置中分离出PRP。正常血液中血小板的含量为125×109/L~350×109/L，离心后的PRP血小板浓度是全血的3倍左右，血小板所含的生长因子浓度相应增加；临床上多使用血液成分分离机和一次性使用单采单采血液成分分离器进行采集PRP。某些因素的刺激下，血小板被激活后会分泌释放PVEs等物质，Aatonen等总结了PVEs的分离过程可分为4个关键步骤：第一步，从全血中提取富血小板血浆；第二步，从PRP中进一步分离血小板；第三步，激活血小板，诱导EVs释放；第四步，通过差速离心法分离EVs[34]。PRP根据血小板、白细胞和纤维蛋白含量的不同分为纯富血小板血浆(P-PRP)、富白细胞和富血小板血浆(L-PRP)、纯富血小板纤维蛋白(P-PRF)、富含白细胞和血小板的纤维蛋白(L-PRF)等[34-36]。在这里，我们提供了PRP、VEs的分类简述[20, 37, 38]。同时，我们还提供了制备PRP、PVEs的简要流程图。

同时，根据PRP的来源不同分为自体PRP、同种异体PRP、脐带血PRP；使用不同类型的PRP可以对疾病进行更精细化的治疗，并取得更好的疗效[39, 40]。近来有研究表明，使用同种异体PRP或脐带血PRP治疗骨关节炎等肌肉骨骼疾病取得了一定的成效[41, 42]；特别是使用同种异体PRP治疗KOA效果显著，Anitua等认为使用自体PRP治疗KOA的效果略胜一筹[43, 44]。然而，对于需要大量输血、糖尿病导致的难愈性创面、新生儿和婴儿、老年人、免疫功能低下或虚弱、血液疾病和癌症等患者难以使用自体PRP治疗[45]，使用同种异体PRP和脐带血PRP治疗这些疾病是临床亟需解决的难点和热点。因此，需要对PRP的分子机制和功能进行全面了解，有助于研发出安全高效、费用低廉的同种异体PRP以及脐带血PRP用于临床治疗疾病，减轻患者的经济负担。值得注意的是，分类标准尚未给出剂量依赖效应的数据，血小板浓缩物中的血小板数量、纤维蛋白和白细胞含量尚未明确，需进一步研究[36, 46]。

**PRP、PEVs的生物学特性**

**PRP的生物学特性**

PRP中富含地生长因子与软骨修复密切相关，如PDGF可调节胶原、蛋白多糖的分泌和合成，促进软骨细胞的增殖；VEGF可调节血管的生成，促进慢性损伤修复和软骨内成骨等。PRP的生物学特性为其在组织损伤修复中的应用提供了理论基础，有文献表明PRP的组成成分存在个体差异，在男性PRP中细胞因子（IL-1β、IRAP、TNF-α）和生长因子（PDGF、VEGF和TGF-β）含量比女性高[47]；Ng and Murphy等人认为：PDGF和FGF在MSCs的增殖和分化中伴有重要角色，并且脐带血PRP中PDGF和FGF含量多于外周血PRP[48, 49]**。**总的来说，使用PRP治疗骨关节炎等肌肉骨骼疾病是可行的，但需要对PRP的分子机制和功能进行深入了解，有助于开发一种高效安全的PRP，特别是脐带血PRP。

**PEVs的特性分析**

最初研究表明存在膜碎片或来源于活化血小板的"富脂颗粒"，被称为"血小板粉尘"，现在被认为是PEVs，具有促凝活性，当血小板附着在血管壁上时，PEVs被释放[50, 51]。现研究表面PEVs是血小板在各种激活刺激产生的膜结构的颗粒混合物，循环中的PEVs数量较大约占血液来源EV总量的一半以上。PEVs在zeta电位上带负电荷，表面有阴离子磷脂、细胞源性抗原、细胞因子和基质金属蛋白酶，内部含有mRNA和microRNA[30]。PEVs表面存在CD31、CD41、CD42a、P-selectin、PF4和GPIIb/IIIa等潜在的标记物[33]。

**PRP在KOA治疗中的应用**

早期KOA患者膝关节内注射的PRP，浸润软骨和滑膜能促进膝关节生物环境的改变，从而缓解临床症状抑制KOA的进展；对于严重KOA患者，软骨下骨板明显增厚，注射的PRP并未触及关节内软骨，对晚期KOA患者治疗效果有限[52]。除了单一的关节内注射外，关节内和关节外联合注射PRP（冠状动脉内侧/内侧副韧带进行单一关节内注射和关节外注射），减轻KOA患者疼痛、促进膝关节功能改善以及提高患者生活质量、身心健康[53]。通过这些研究就表明，PRP治疗KOA的效果除了与PRP本身的种类和质量相关[54, 55]，还与注射部位、注射量、以及注射次数相关[36, 44, 46]。值得注意的是，制备一次的PRP可分装储存于-80℃冰箱中，用于多次注射治疗，减少患者的抽血次数以及治疗费用，还显著提高了患者满意度[56]。

近年多项研究证实PRP对于关节软骨的修复作用，Zhu等研究结果显示，在轻/中度骨关节炎的髋关节和膝关节的关节腔内注射透明质酸( HA )和PRP，AS (Visual Analogue Scale) 和 WOMAC (Western Ontario and McMaster Universties Osteoarthritis Index) 等专科评分显著提高，关节功能得到明显的改善[57]。Qi等研究表明，PRP中的生长因子HGF和IGF-1调控软骨细胞NF-kB信号通路抑制IL-1β活化，进而阻断炎症的发生发展[58]。此外，PRP能够抑制阿霉素诱导的小鼠关节软骨细胞中IκB和NF-κB的磷酸化，减少体内软骨破坏[59]。这些研究表明PRP修复软骨缺损的潜在机制，为临床应用PRP治疗KOA提供了理论依据。到目前为止，在著名骨科期刊上发表与PRP相关的文献占45%左右，PRP在骨科疾病的疗效仍有争议；由于研究样本量不足、证据水平低、系统命名混乱、基础研究不足、制备方法缺乏标准化、注射部位、注射量、注射次数及间隔时间与治疗效果相关等诸多问题[60-62]，。如上所述，PRP由于其方便、安全、高效的性能，被广泛应用于各种临床领域，促进组织修复和再生。PRP在再生医学中的作用机制尚未完全了解，以往的普遍观点认为，PRP强大的修复能力主要来源于大量分泌的生长因子；然而，最近的研究表明，除了生长因子外，PRP中血小板被激活后还会释放大量的PEVs，参与组织修复的调控[63]。

**PEVs在KOA治疗中的应用**

活化的血小板释放的细胞外囊泡（PEVs）有外泌体和微囊泡两种亚型[64]，PEVs体积较小可到达体内各部位发挥其功能，免疫原性低、能够在局部释放、且容易获得等优点正受到临床的关注[65]。大量研究表明，PEVs可调节软骨细胞外基质的代谢和炎症反应，诱导成软骨基因表达，减少促炎细胞因子的释放抑制炎症的发生发展[66]；PEVs通过抑制巨噬细胞产生促炎因子TNF-α、IL-8等减少炎症反应[67, 68]；也有学者认为PEVs介导的脂质、IL-1β等损伤相关分子促进炎症信号转移[69]；Liu 等报道了PEVs促进巨核细胞分化和血栓生成以及血管生成和组织再生的能力[70]。此外，在一项临床前研究中，PEVs被用于延缓糖皮质激素诱导的股骨头坏死的疾病进展[71]。值得注意的是，细胞外泌体通过Akt/Bad/Bcl-2信号通路抑制内质网应激诱导的细胞凋亡，缓解大鼠股骨头坏死[72]；外泌体激活Wnt/β-catenin信号通路抑制IL-1β诱导的软骨细胞降解和炎症反应，从而缓解KOA的临床症状[17]。这些研究表明PEVs修复软骨缺损和抑制炎症的潜在机制，为临床应用PEVs治疗KOA提供了理论依据。鉴于这些研究发现，为了使PEVs在临床上治疗疾病更加安全有效，应充分挖掘其潜在功能；特别是PEVs制备方法、研究样本量、基础研究等诸多问题仍需改进。

Antich等报道PEVs是间充质干细胞成骨分化的重要效应因子，并呈剂量依赖性[73]；当使用不同的激动剂激活血小板产生PEVs时，PEVs的数量、细胞因子的含量以及生物学作用是不同的。Rui等报道，葡萄糖酸钙和凝血酶混合物激活血小板释放的PEVs浓度最高，其次是钙激活组，然后是凝血酶激活组[74]，对于不同激活剂的差异机制以及影响因素尚不明确，需进一步探索。

**展望**

尽管目前PRP/PEVs治疗KOA的临床研究尚处于起步阶段，仍需大样本临床试验进一步验证其疗效，鉴于目前PRP/PEVs大量的细胞及动物试验的研究成果[75-77]，PRP/PEVs有望成为促进KOA修复的新方法，从而为临床治疗KOA提供更为广阔的应用前景。

PRP/PEVs具有促进组织再生修复的潜能，被广泛应用于再生医学，对于KOA再生修复进行新的探索，初步显示出良好的疗效[78-80]。但是，仍然存在一些问题需要解决。首先，由于PRP本身成分复杂，其所释放的多种细胞因子促进组织再生修复的确切机制仍需进一步阐明；其次，由于目前对PRP/PEVs的制备方案及有效治疗浓度缺乏统一共识，不同制备方法产出的PRP中血小板及细胞因子的浓度亦有所不同，使得不同研究呈现出的临床疗效不尽相同，且现有临床研究多为小样本、缺乏长期随访的数据。最后，PRP/PEVs注射部位、注射剂量、注射时长及周期、不良反应以及病人的自身条件等因素使得临床疗效不尽相同；随着对PRP/PEVs研究的不断深入，未来需建立标准的PRP/PEVs制备方案，探索最佳治疗浓度、剂量及周期等，并进行大规模的临床随机对照试验以及进一步观察PRP/PEVs的短期、长期疗效。此外，如将异体/脐带血PRP与PEVs相结合，将有望成为一种新的治疗KOA以及其他疾病的新方法，服务于更多的临床患者。

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