



### 富血小板血浆的三次离心法制备及其对脂肪干细胞增殖、成骨矿化的影响

Platelet-rich plasma generated via the three centrifugalization method and its impact on adipose tis

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**摘要** 目的 探索高效制备富血小板血浆 (PRP) 的方法, 并观察不同体积分数的PRP 对Beagle 犬脂肪干细胞 (ADSC) 体外增殖及其向成骨细胞分化的影响。方法 抽取3 只Beagle 犬静脉血各40 mL, 分别用传统的二次离心法和改良的三次离心法制备PRP, 进行血小板计数分析。分别将体积分数为5%、10%、15%、20%的PRP 加于Beagle 犬ADSC 的培养液中, 于作用后48、72、96 h 检测细胞的增殖状况; 将ADSC 分别培养在添加了体积分数为5%、10%、15%、20%的PRP 和不加PRP 的成骨细胞诱导液中, 观察ADSC 的成骨表达以及PRP 对其成骨矿化的影响。结果 三次离心法制备的PRP 中血小板的含量明显高于二次法制备的。各组PRP 促ADSC 增殖作用均显著强于阴性对照组 ( $P<0.001$ ); 不同体积分数PRP 组间差异有统计学意义 ( $P<0.001$ ), 细胞增殖在体积分数15%PRP 组、96 h 时达到峰值。von Kossa 染色中矿化结节数量随着浓度上升而增多, 体积分数20%PRP 组最多。结论 三次离心法制备PRP 的效率高于二次离心法。PRP 对Beagle 犬ADSC 的体外增殖有促进作用, 与PRP 体积分数和作用时间呈正相关关系。ADSC 矿化结节的数量与PRP 的体积分数呈正相关关系。

**关键词:** 富血小板血浆 脂肪干细胞 细胞增殖 成骨矿化

**Abstract:** Objective The study investigated an effective method of generating platelet-rich plasma (PRP) and evaluated the impact of different concentrations of PRP on adipose tissue-derived stem cell (ADSC) proliferation and mineralization *in vitro*. Methods 40 mL whole blood was collected from 3 healthy Beagle dogs and then PRP was separated by the two centrifugalization method and the three centrifugalization method respectively. Then the platelet was counted and analyzed. ADSC were exposed to DMEM medium with various concentrations of PRP (5%, 10%, 15%, 20%) respectively. After incubation of 48, 72, 96 h, the proliferations of ADSC were measured. ADSC were cultured in osteoblasts induced medium and osteoblasts induced medium with PRP (5%, 10%, 15%, 20%) respectively, The osteogenetic expression of ADSC and the influence on its osteogenesis mineralization of PRP were observed. Results The number of platelet from the three centrifugalization method was more than that from the two centrifugalization method. The role on various concentrations of PRP on cell proliferation was more significant than that of negative control ( $P<0.001$ ), the differences between various concentrations of PRP groups had a statistical significance ( $P<0.001$ ). The group of 96 h, 15% PRP reached to peak value in ADSC proliferation. The quantity of mineralization node in ADSC was maximized under the condition of osteoblasts induced medium with 20% PRP. Conclusion The three centrifugalization method was more efficient than the two centrifugalization method. PRP promoted ADSC proliferation in both the dose- and time- dependent ways while PRP improved ADSC mineralization in the dose-dependent way only.

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