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MC3T3-E1 前成骨细胞培养时间对矿化结节表达的影响 *

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摘要 目的：观察 MC3T3-E1 前成骨细胞不同培养时间点矿化结节的形态，探讨一个既节省实验时间与经费，又便于观察矿化结节形态差异的实验方法。**方法：**将 MC3T3-E1 前成骨细胞按培养时间分为四组（14、21、28、35 天组），各组实验结束时行茜素红染色，光学显微镜下观察矿化结节的形态变化。**结果：**各组均见红色的矿化结节形成，随培养时间延长，染色面积增大，密度增高，14 天时结节轮廓清晰，结节间距较大，21 天时结节面积增大，28 天时结节边界超出视野，35 天时视野内大片深染，结节轮廓不清。**结论：**在本实验周期内，MC3T3-E1 前成骨细胞培养 14 至 21 天通过茜素红染色可以较清晰地观察矿化结节，其中培养 14 天时即可观察到结节大小、数量及形态，考虑到实验时间及经费的因素，我们认为 MC3T3-E1 前成骨细胞培养 14 天后行茜素红染色是观察不同因素对其矿化产生影响的适宜时间点。

关键词：MC3T3-E1；培养时间；矿化结节；茜素红染色

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Effects of Culture Time of MC3T3-E1 Pre-osteoblasts on the Expression of Mineralized Nodules*

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ABSTRACT Objective: To observe the effect of MC3T3-E1 pre-osteoblasts at different culture time on mineralized nodules morphology, and explore an experimental method which not only can save experiment time and funds, but also is convenient to observe the morphology differences of mineralized nodules. **Methods:** The MC3T3-E1 pre-osteoblasts were divided into four groups according to the cultured time (14, 21, 28, 35 days group), then they were stained by alizarin red staining at the end of the experiment, to observe the morphological changes of the mineralized nodule under optical microscope. **Results:** The formation of mineralized nodule was detected in all the four groups. With the extension of the cultured time, the staining area increased, and the density also increased. On the 14th day, the nodules were clear, and the distance between nodules was large, and on the 21st day, the area of nodules increased, and on the 28th day, the nodule boundary were beyond vision, while on the 35th day, the field was largely hyperchromatic, and the outline of nodule was unclear. **Conclusion:** In the period of this experiment, it was suitable for MC3T3-E1 pre-osteoblasts to culture for 14 to 21 days by alizarin red staining. Cultured for 14 days, the cell number, size and morphology can be clearly observed when it is the best time to study the effects of different factors on the MC3T3-E1 pre-osteoblast mineralization.

Key words: MC3T3-E1; Culture time; Mineralized nodules; Alizarin red staining

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前言

骨在人体中发挥重要的作用，为了行使其相应功能，骨组织持续进行改建，即骨组织形成和骨组织吸收。成骨细胞是骨形成细胞，可分泌细胞外基质，参与后续的骨矿化过程[1]。

MC3T3-E1 前成骨细胞是目前国内外广泛应用的成骨细胞系，经体外培养后行茜素红染色，可观察矿化结节的大小、数量及形态，反映细胞的矿化能力。但观察矿化结节的培养时间差异较大，从 8 天至 30 天不等，其中 14[2]、21[3]、28 天[4]应用较多。故本实验拟通过对 MC3T3-E1 前成骨细胞不同培养时间点矿化结节的观察，探讨一个既便于观察矿化结节大小、数量及形态，又节省实验时间与经费的实验方法。

1 材料和方法

1.1 材料及仪器

MC3T3-E1 前成骨细胞（ATCC，美国），α-MEM 培养基、胎

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牛血清和青链霉素(Gibco,美国),抗坏血酸、β- 甘油磷酸钠和茜素红(Sigma,美国),二氧化碳培养箱(Heal Force,中国),倒置显微镜 (Olympus, 日本),Nikon ECLIPSE 80i 光学显微镜,Nikon DS-Ri1 采集系统,NIS-Elements BR3.0 图文处理系统(尼康,日本)。

1.2 细胞培养

MC3T3-E1 前成骨细胞接种于含 α-MEM 培养液(10 %胎牛血清,1 %青链霉素)的培养瓶内,放入 CO_2 孵育箱(37 ℃,5 %CO_2,潮湿)培养,3 天更换一次培养液,待细胞铺满瓶底可用胰蛋白酶消化,进行传代或冻存,用于后续实验。

24 孔板每孔放入同样大小的盖玻片, 将 MC3T3-E1 前成骨细胞按每孔约含 1 万细胞数接种到盖玻片上,加诱导液(培养液中含 50 μg/mL 抗坏血酸和 10 mmol/L β- 甘油磷酸钠)放入 CO_2 孵育箱继续培养,三天更换一次诱导液。

1.3 茜素红染色

细胞培养至第 14、21、28、35 天时分别行茜素红染色:去掉旧液体,PBS 冲洗细胞层 2 次,95 %乙醇固定 10 min,双蒸水冲洗细胞,0.1 %茜素红 -Tris-Hcl(pH 8.3)37 ℃ 染色 30 min,双蒸水冲洗后,即可见红色的矿化结节[5],将盖玻片取出,常规封片可长期保存观察。

2 结果

MC3T3-E1 前成骨细胞茜素红染色各实验组均见红色矿化结节形成,随着实验时间的延长,染色面积逐渐增大,密度增高。低倍镜下观察结节形态:14 天时视野内见两个矿化中心,结节呈梭形,面积较小,密度较低,两结节间距较大;21 天时结节呈椭圆形,面积增大,密度增高;28 天时结节范围进一步增大,边缘超出视野,局部呈点片状深染;35 天时视野内大片深染,密度增高,难以分辨矿化中心及结节轮廓(如图 1)。

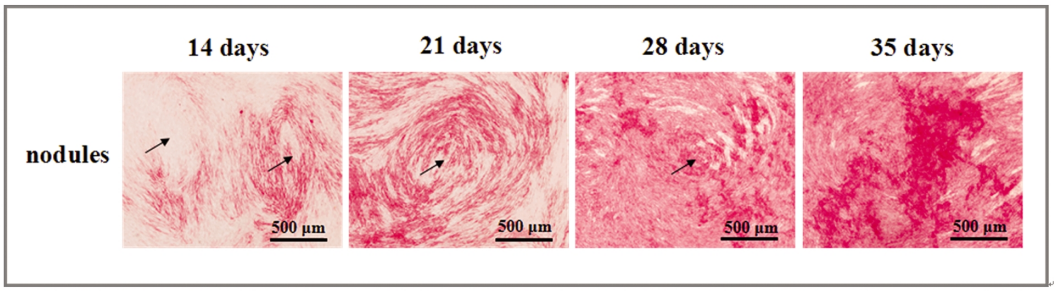

图 1 MC3T3-E1 前成骨细胞培养不同时间茜素红染色图

Fig. 1 Alizarin red staining of MC3T3-E1 pre-osteoblast culture for different time

3 讨论

MC3T3-E1 前成骨细胞是 Kadama 等利用新生小鼠颅顶骨建立的克隆细胞系[8],在条件培养基(α-MEM 培养液含 10 %胎牛血清、1 %青链霉素、50 μg/mL 抗坏血酸和 10 mmol/L β- 甘油磷酸钠)中培养[6]具有体外增殖、分化及矿化能力[8],是目前体外研究成骨的常用模型[7]。MC3T3-E1 前成骨细胞在对数生长期细胞形态呈成纤维细胞样, 细胞质中含丰富的微丝和微管。当细胞汇合以后,细胞间互相接触外观似 " 马赛克 " 样[8]。随着培养时间的延长,细胞可分化为成骨细胞,此时期的细胞连接紧密排列呈 " 漩涡 " 样。

根据 MC3T3-E1 骨相关蛋白表达时间不同,可将细胞培养过程分为增殖期(4～10 天)、骨基质形成 / 成熟期(10～16 天)和矿化期(16～30 天)[10]。早期Ⅰ型胶原表达、合成并分泌到基质中,10 天时碱性磷酸酶、纤连蛋白、TGF-β1 和骨粘连蛋白表达逐渐增多,基质开始矿化发生于 2 周左右,随着培养时间的延长,矿化结节的大小及数量逐渐增加[8]。MC3T3-E1 前成骨细胞骨相关蛋白的表达以时间依赖方式,并影响基质矿化[9,10]。矿化期的细胞叠加生长层次增多,钙盐在成骨细胞膜、基质小泡、未成熟的骨细胞和富含溶酶体的细胞中表达明显[8]。矿化结节是矿化基质形成的特征性指标,是成骨细胞体外成骨表型的最终表达[11]。茜素红能选择性的与钙离子结合[12],应用此染色法识别矿化结节较为灵敏,染色后的矿化结节呈红色,肉眼即可观察[13]。基于以上文献回顾,我们选择细胞培养 14 天作为初始时间,茜素红染色作为实验方法,于培养 14、21、28、35 天时行茜素红染色观察矿化结节。

在本实验周期内,MC3T3-E1 前成骨细胞经不同培养时间行茜素红染色,低倍镜下观察矿化结节的形态,可见 14 天时结节呈梭形,面积较小,两结节间轮廓清晰;21 天时结节呈椭圆形,面积增大,密度增高;28 天时结节边缘已超出视野,局部呈点片状深染;35 天时视野内大片深染,难以分辨结节轮廓。根据以上结果分析,MC3T3-E1 前成骨细胞培养 14 天至 21 天通过茜素红染色观察矿化结节较清晰,考虑到实验时间及经费的因素,我们认为培养 14 天时即可清晰地观察矿化结节的大小、数量及形态,是研究不同因素对该细胞矿化产生影响的适宜时间点。

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