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· 基础研究 ·

# LED红光对人根尖乳头干细胞增殖和成骨分化的影响

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**【摘要】** 目的 探讨LED红光对人根尖乳头干细胞(human stem cells from apical papilla, hSCAPs)增殖和成骨分化的影响。方法 hSCAPs通过体外分离培养和流式鉴定获得,分别用1、3、5、7 J/cm<sup>2</sup> LED红光照射。CCK-8检测细胞增殖。碱性磷酸酶(alkaline phosphatase, ALP)染色及定量活性检测、茜素红定量检测分析成骨分化。RT-PCR实验和Western blot实验分别检测5 J/cm<sup>2</sup> LED红光对hSCAPs中ALP、Runx2相关转录因子2(Runt-related transcription factor 2, Runx2)、骨钙素(osteocalcin, OCN)、骨桥蛋白(osteopontin, OPN)和骨唾液酸蛋白(bone sialoprotein, BSP)基因及蛋白表达水平的影响。结果 1、3、5、7 J/cm<sup>2</sup> LED红光照射促进hSCAPs的增殖( $P < 0.05$ );不同能量LED红光在不同的时间点照射对hSCAPs增殖的促进作用有差异( $P < 0.05$ );在成骨诱导培养下,光照后第7天和第14天,LED红光照射促进hSCAPs的成骨分化,且5 J/cm<sup>2</sup> LED红光照射促进作用最明显( $P < 0.05$ );5 J/cm<sup>2</sup> LED红光照射上调SCAPs中ALP、Runx2、OCN、OPN和BSP基因及蛋白的表达( $P < 0.05$ )。结论 LED红光照射促进hSCAPs增殖和成骨分化。

**【关键词】** 间充质干细胞; 人根尖乳头干细胞; 光生物调节; LED红光; 细胞增殖; 碱性磷酸酶; 骨钙素; 骨桥蛋白; 成骨分化; 组织工程; 干细胞治疗

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**Effects of a red light-emitting diode on the proliferation and osteogenic differentiation of stem cells from the apical papilla** SU Yutong, HOU Lan, JIANG Bing, ZHENG Genzi, LIU Yuan, WANG Yao. The Affiliated Stomatology Hospital of Southwest Medical University, Luzhou 646000, China

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**【Abstract】 Objective** To explore the effects of red LEDs on the proliferation and osteogenic differentiation of human stem cells from apical papilla (hSCAPs). **Methods** hSCAPs were obtained by isolation, culture and flow cytometry in vitro and irradiated with 1, 3, 5, and 7 J/cm<sup>2</sup> red LEDs. The proliferation of hSCAPs was detected using a CCK-8 assay. The osteogenic differentiation of hSCAPs was evaluated using alkaline phosphatase (ALP) staining, ALP activity assay and Alizarin red quantitative detection. The effect of 5 J/cm<sup>2</sup> red LEDs on the expression levels of the ALP, Runx2, OCN, OPN and BSP genes and proteins was detected by RT-PCR and western blot, respectively. **Results** Red LEDs at 1, 3, 5, and 7 J/cm<sup>2</sup> promoted the proliferation of hSCAPs ( $P < 0.05$ ). The effects of red LEDs with different light energies on the proliferation of hSCAPs were different at different time points ( $P < 0.05$ ). On the 7th and 14th days after irradiation, red LEDs promoted the osteogenic differentiation of hSCAPs, and the effect of 5 J/cm<sup>2</sup> red LEDs was the most obvious under osteogenic induction culture conditions ( $P < 0.05$ ). Red LEDs (5 J/cm<sup>2</sup>) promoted the expression of the ALP, Runx2, OCN, OPN and BSP genes and proteins ( $P < 0.05$ ). **Conclusion** Red LEDs promoted the proliferation and osteogenic differentiation of hSCAPs.

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**【Key words】** mesenchymal stem cells; human stem cells from apical papilla; photobiomodulation; red LED; cell proliferation; alkaline phosphatase; osteocalcin; osteopontin; osteogenic differentiation; tissue engineering; stem cell-based therapy

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**【Competing interests】** The authors declare no competing interests.

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牙源性干细胞是目前的研究热点,目前已经被分离和鉴定的人类牙源性干细胞有5种<sup>[1]</sup>。根尖乳头干细胞(human stem cells from apical papilla, hSCAPs)是由 Sonoyama 等从成年人的牙齿中分离出来的一组具有干细胞特性的细胞群,具备多向分化能力<sup>[2-3]</sup>。hSCAPs 较其他牙源性干细胞具备更强的自我更新和分化能力<sup>[4]</sup>,其增殖和分化可以促进牙根发育。hSCAPs 是干细胞治疗中潜在的细胞来源<sup>[5]</sup>。

光生物调节(photobiomodulation, PBM)是通过低能量激光或发光二极管(light-emitting diode, LED)照射来调节生物组织功能,可以有效减少炎症<sup>[6]</sup>、促进伤口愈合<sup>[7]</sup>、增强细胞活力<sup>[8]</sup>、促进细胞增殖和成骨分化能力<sup>[9]</sup>。LED 红光是指波长在 620~720 nm 范围内的不相干光源,穿透力强,可以达到组织深层。LED 红光较激光具有仪器便于携带、经济、安全的特点<sup>[7]</sup>,能促进干细胞增殖和成骨分化<sup>[10]</sup>。LED 红光对牙源性干细胞生物学特性的影响机制尚不明确。因此本实验选择 hSCAPs 为研究对象,探讨 LED 红光照射对其增殖和成骨分化的影响。

## 1 材料和方法

### 1.1 主要试剂与仪器

CCK-8 试剂盒(Dojindo, 日本),低糖 DMEM (Hyclone 公司,美国),青霉素-链霉素(Bioder 公司,中国),胎牛血清(四季青,中国),Dispase II 分散酶(Yeasen, 美国),PBS 磷酸盐缓冲液干粉、胰蛋白酶、地塞米松、 $\beta$ -甘油磷酸钠、维生素 C、1% Triton-X100、茜素红染色液、氯化十六烷吡啶(Sloarbio 公司,中国),抗 CD24、抗 CD34、抗 CD45、抗 CD90、抗 CD146 (Ebioscience, 美国),I 型胶原酶、多聚甲醛(Biosharp 公司,中国),碱性磷酸酶显色试剂(生工公司,中国),BCA 蛋白浓度测定试剂盒(Beyotime 公司,中国),碱性磷酸酶测定试剂盒(南京建成,中

国),TRIpure Total RNA Extraction Reagent、EntiL-ink™ 1st Strand cDNA Synthesis Kit、EnTurbo™ SYBR Green PCR SuperMix(ELK Biotechnology, 中国)。

生物安全柜(BSC-1000 II A2, 苏州净化设备厂,中国),低速离心机(KDC-1044, 中佳,中国),CO<sub>2</sub> 孵箱(CCL-170B-8, Esco Micro Pte. Ltd., 新加坡),流式细胞仪(FACS Aria, BD, 美国),倒置相差荧光显微镜(IX2-ILL100, Olympus, 日本),LED 红光灯(T6, 芮森, 中国),高精度光功率计(Field-MaxII-TO, Coherent, 美国),酶标仪(Synergy™ HTX, BioTek, 美国),PCR 仪(Veriti 96-Well Thermal Cycler, Thermofisher, 新加坡),荧光定量 PCR 仪(CFX Connect™, Bio-Rad, 美国),蛋白转膜系统(DYY-6C, 北京市六一仪器厂,中国)。

### 1.2 hSCAPs 的分离培养和鉴定

本实验已取得西南医科大学附属口腔医院伦理委员会批准(批号:20180314001)。根尖乳头组织来源于西南医科大学附属口腔医院颌面外科。患者因正畸需拔除的阻生第三磨牙,获得患者及家属知情同意后立即放入 PBS 中备用。待生物安全柜消毒完毕后,用含有 20%、3% 青霉素-链霉素双抗的 PBS 反复冲洗净组织表面残留物,将组织剪碎,加入 I 型胶原酶(3 g/L)和 Dispase 酶(4 g/L),待组织块消化至絮状后加入等量完全培养基中止反应,将组织块接种于培养瓶中,加入含 10% FBS 的低糖 DMEM 培养基,于 37 °C、5% CO<sub>2</sub> 条件下培养,每隔 3 d 换液,细胞长满瓶底后传代。取第 3 代 SCAPs,经成骨诱导及成脂诱导培养 3 周后,去除上清液,PBS 清洗 3 遍,多聚甲醛固定 30 min。PBS 清洗 2 遍,进行茜素红染色及油红 O 染色。取第 3 代 SCAPs, PBS 重悬,分别加入抗人 CD24、CD34、CD45、CD90、CD146 抗体,室温避光孵育 30 min,流式细胞仪检测细胞表面标志物。

### 1.3 实验分组及干预

本研究采用的 LED 红光光源功率输出稳定、

连续,波长在600~700 nm之间。光源与细胞之间的距离为2 cm。在这些条件下,测得光源的功率密度约为66.7 mW/cm<sup>2</sup>。根据公式:辐射曝光量(radiant exposure, J/cm<sup>2</sup>)=功率密度(power density, W/cm<sup>2</sup>)×时间(irradiation time, s)计算可得,LED红光照射15、45、75、105 s时,辐射曝光量分别为1、3、5、7 J/cm<sup>2</sup>。将细胞分为0 J/cm<sup>2</sup>组(对照组),1 J/cm<sup>2</sup>组,3 J/cm<sup>2</sup>组,5 J/cm<sup>2</sup>组和7 J/cm<sup>2</sup>组,每48 h对细胞进行一次照射。所有细胞均在暗室中进行照射,照射的第一天设为光照后第0天。

#### 1.4 CCK-8检测LED红光对hSCAPs增殖的影响

取第4代hSCAPs接种于96孔板,密度为2×10<sup>3</sup>/孔,每组设置5个副孔,加入含10% FBS的低糖DMEM培养基,37℃、5% CO<sub>2</sub>条件下孵育。次日换液后分组进行光照。分别在光照后的第1、3、5、7、9天进行CCK-8检测。去原培养液,加入CCK-8混合液,孵育1 h,酶标仪(450 nm)检测吸光度值。根据每个时间点的平均吸光度绘制细胞生长曲线。

#### 1.5 ALP染色和ALP定量检测hSCAPs成骨分化

取第4代hSCAPs接种于3.5 cm细胞培养皿中,加入含10% FBS的低糖DMEM培养基、37℃、5% CO<sub>2</sub>孵育,2 d后更换为成骨诱导培养基。分组进行光照。培养至第7、14天时,去上清液,PBS清洗3遍,多聚甲醛固定30 min。PBS清洗2遍,根据ALP显色试剂说明书配置染液,染色15 min。在倒置显微镜下观察细胞。

hSCAPs经成骨诱导培养至第7、14天时,胰蛋

白酶消化,1% TritonX-100裂解细胞40 min。使用BCA蛋白浓度测定试剂盒测定裂解物中总蛋白质浓度、ALP测定试剂盒测定样本细胞内ALP活性,计算ALP相对活性。

#### 1.6 茜素红定量检测hSCAPs成骨矿化

hSCAPs经成骨诱导培养至第21天时,去除上清液,PBS清洗3遍,多聚甲醛固定30 min。PBS清洗2遍,1%茜素红染色5 min。超纯水清洗2遍,加入氯化十六烷基吡啶溶液,室温避光静置30 min。将溶解后的上清液分别置于96孔板中,每组设置5个副孔,酶标仪(562 nm)检测吸光度值。

#### 1.7 RT-PCR检测hSCAPs中成骨相关基因的表达

选择5 J/cm<sup>2</sup> LED红光照射条件作为RT-PCR的实验组。hSCAPs经成骨诱导培养至第7、14天时,使用TRIpure Total RNA Extraction Reagent试剂盒进行总RNA提取。使用EntiLink™ 1st Strand cDNA Synthesis Kit试剂盒进行第一链cDNA的合成。以GAPDH为内参照,使用EnTurbo™ SYBR Green PCR SuperMix试剂盒进行实时聚合酶链反应(RT-PCR)分析各组细胞中成骨相关基因碱性磷酸酶(alkaline phosphatase, ALP)、骨钙素(osteocalcin, OCN)、骨桥蛋白(osteopontin, OPN)、骨唾液酸蛋白(bone sialoprotein, BSP)和Runt相关转录因子2(Runt-related transcription factor 2, Runx2)的表达水平,每个样品均设置3个复孔。目的基因的相对表达量通过2<sup>-ΔΔCT</sup>方法计算得出。相关基因的引物序列见表1。

表1 成骨相关基因引物序列

Table 1 Primer sequences of osteogenesis-related genes

Gene	Forward primer sequence	Reverse primer sequence
ALP	GGACGATGGCTCTGATGACC	GGTTTCGCAGTACAGCTCCC
OCN	CACACTCCTCGCCCTATTGG	GATGTGGTCAGCCAACCTCGTC
OPN	GTACCCTGATGCTACAGACGAGG	CTCGTTTCATAACTGTCTTCCC
BSP	GGGGTCTTTAAGTACAGGCCA	GCCCAGTGTGTAGCAGAAAGT
Runx2	GGAGTGGACGAGGCAAGAGTT	TGGTGCAGAGTTCAGGGAGG
GAPDH	CATCATCCCTGCCTCTACTGG	GTGGGTGCTCGCTGTGAAGTC

ALP: alkaline phosphatase; OCN: osteocalcin; OPN: osteopontin; BSP: bone sialoprotein; Runx2: Runt-related transcription factor 2

#### 1.8 Western blot检测hSCAPs中成骨相关蛋白的表达

成骨诱导培养至第7、14天时,收集对照组和5 J/cm<sup>2</sup>组细胞,使用RIPA总蛋白裂解液提取细胞总蛋白,BCA蛋白质浓度测定试剂盒测定裂解物中总蛋白质浓度,进行十二烷基硫酸钠聚丙烯酰胺凝胶电泳(SDS-PAGE),转膜后,加入封闭液室

温封闭1 h,孵育一抗,二抗,增强化学发光(enhanced chemiluminescence, ECL)方法进行显影。

#### 1.9 统计学分析

采用SPSS 17.0进行统计学分析。结果进行方差齐性检验,Shapiro-Wilk法进行正态性检验,采用重复测量数据的方差分析进行多组间的比较,LSD法进行不同组间样本均数的两两比较。计量资料

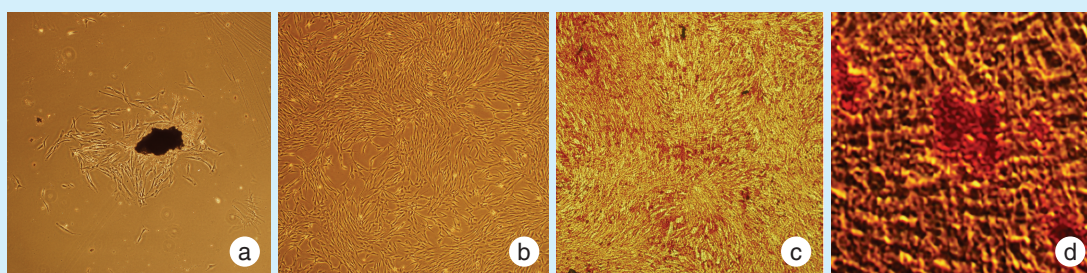
以均数±标准差表示,  $P < 0.05$  为差异有统计学意义。

## 2 结果

### 2.1 hSCAPs 的分离培养和鉴定

5 d后可见细胞从组织块边缘爬出, 贴壁生长, 呈长梭形, 见图 1a。培养约 20 d, 可见细胞趋

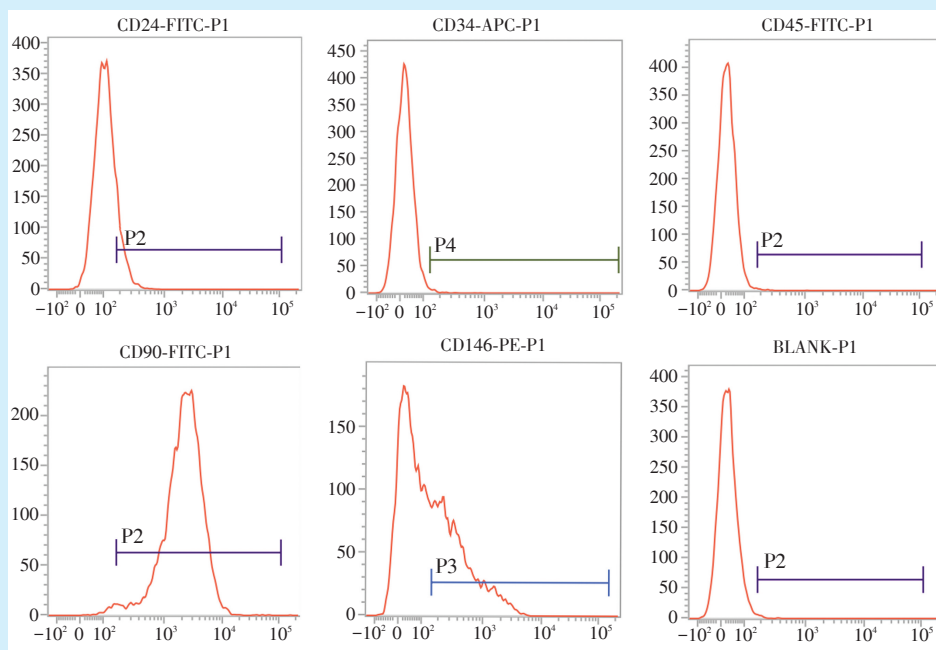
于融合, 见图 1b; 成骨诱导 3 周后, 茜素红染色可见钙结节沉积, 见图 1c; 成脂诱导 3 周后, 油红 O 染色可见着色的脂滴, 见图 1d。流式细胞鉴定结果: 第 3 代 hSCAPs 的干细胞表面标志物 CD24、CD90、CD146 表达呈阳性, 造血干细胞表面标志物 CD34、CD45 呈阴性, 表明获得的细胞属于间充质干细胞谱系, 见图 2。



a: primary hSCAPs crawled out from the edge of tissue on day 5 ( $\times 40$ ); b: the cells tend to fuse on day 20 ( $\times 40$ ); c: results of alizarin red staining ( $\times 40$ ); d: results of oil red O staining ( $\times 100$ )

Figure 1 Isolation and culture of human stem cells from apical papilla

图 1 人根尖乳头干细胞的分离培养



The expression rates of CD24, CD34, CD45, CD90, and CD146 in SCAPs were respectively 15.57%, 0.81%, 0.35%, 98.70%, 42.42%

Figure 2 Flow cytometry identification of human stem cells from apical papilla

图 2 流式细胞仪鉴定人根尖乳头干细胞

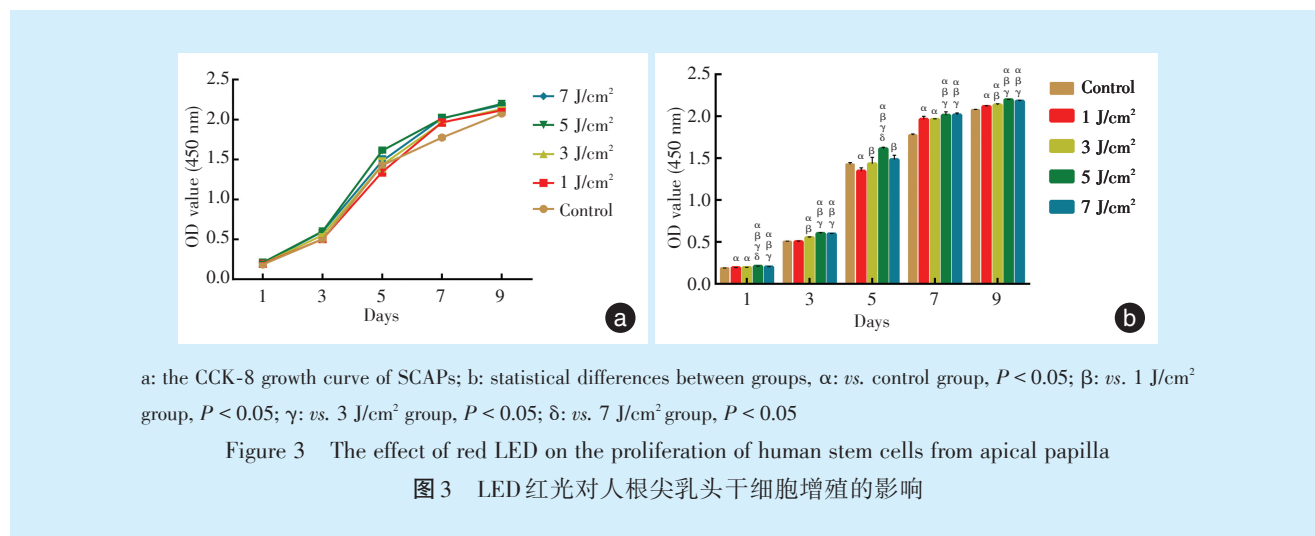
### 2.2 CCK-8 检测 LED 红光对 SCAPs 增殖的影响

根据 CCK-8 检测的结果绘制每组 hSCAPs 的生长曲线, 见图 3a。与对照组相比, 在光照后第 1、3、5、7 和 9 天, 实验组均显著促进 hSCAPs 的增殖。光照后第 1 天 ( $1 \text{ J/cm}^2$  组 vs. 对照组,  $P = 0.002$ ;  $3 \text{ J/cm}^2$

组 vs. 对照组,  $P = 0.001$ ;  $5、7 \text{ J/cm}^2$  组 vs. 对照组,  $P < 0.001$ )、第 7 天 ( $F = 51.110, P < 0.001$ )、第 9 天 ( $F = 83.743, P < 0.001$ ), 各光照组的细胞增殖能力均强于对照组。光照后第 3 天,  $3、5、7 \text{ J/cm}^2$  组的细胞增殖率明显高于对照组 ( $F = 106.942, P < 0.001$ )。光

照后第5天,仅5 J/cm<sup>2</sup>组显示出更强的细胞增殖能力( $F = 18.199, P < 0.001$ )。此外,在各检测时间点

的光照组之间,hSCAPs的细胞增殖率也有差异,见图3b。



### 2.3 ALP染色和ALP定量检测LED红光对hSCAPs成骨分化的影响

LED红光照射后第7天和第14天,ALP染色结果显示光照组较对照组着色较明显,且5 J/cm<sup>2</sup>组着色最深;同时,光照后第14天各组较第7天染色深,见图4a。ALP活性检测结果显示在光照后第7天和第14天,光照组ALP活性高于对照组;光照后第7天,3、5和7 J/cm<sup>2</sup>组促进hSCAPs的ALP活性( $F = 9.380, 3 \text{ J/cm}^2 \text{ 组 vs. 对照组}, P = 0.006; 5 \text{ J/cm}^2 \text{ 组 vs. 对照组}, P < 0.001; 7 \text{ J/cm}^2 \text{ 组 vs. 对照组}, P = 0.008$ );在各光照组之间,5 J/cm<sup>2</sup>组ALP活性最高(5 J/cm<sup>2</sup>组 vs. 1 J/cm<sup>2</sup>组,  $P = 0.002; 5 \text{ J/cm}^2 \text{ 组 vs. } 3 \text{ J/cm}^2 \text{ 组}, P = 0.043; 5 \text{ J/cm}^2 \text{ 组 vs. } 7 \text{ J/cm}^2 \text{ 组}, P = 0.032$ );光照后第14天,仅3 J/cm<sup>2</sup>和5 J/cm<sup>2</sup>组促进hSCAPs的ALP活性( $F = 36.867, 3 \text{ J/cm}^2 \text{ 组 vs. 对照组}, P = 0.035; 5 \text{ J/cm}^2 \text{ 组 vs. 对照组}, P < 0.001$ ),5 J/cm<sup>2</sup>组较其他光照组显著提高ALP活性( $P < 0.001$ );同时,光照后第14天各组都显示出比第7天更高的ALP水平,见图4b。

### 2.4 茜素红定量检测LED红光对hSCAPs矿化的影响

茜素红染色结果见图5a。茜素红定量检测结果( $F = 8.763$ )为仅5 J/cm<sup>2</sup>组促进hSCAPs的矿化( $P < 0.001$ ),且5 J/cm<sup>2</sup>组的矿化结节表达量高于1 J/cm<sup>2</sup>、3 J/cm<sup>2</sup>和7 J/cm<sup>2</sup>组(5 J/cm<sup>2</sup>组 vs. 1 J/cm<sup>2</sup>组,  $P = 0.002; 5 \text{ J/cm}^2 \text{ 组 vs. } 3 \text{ J/cm}^2 \text{ 组}, P = 0.004; 5 \text{ J/cm}^2 \text{ 组 vs. } 7 \text{ J/cm}^2 \text{ 组}, P = 0.001$ )。用1、3、5和7 J/cm<sup>2</sup>LED

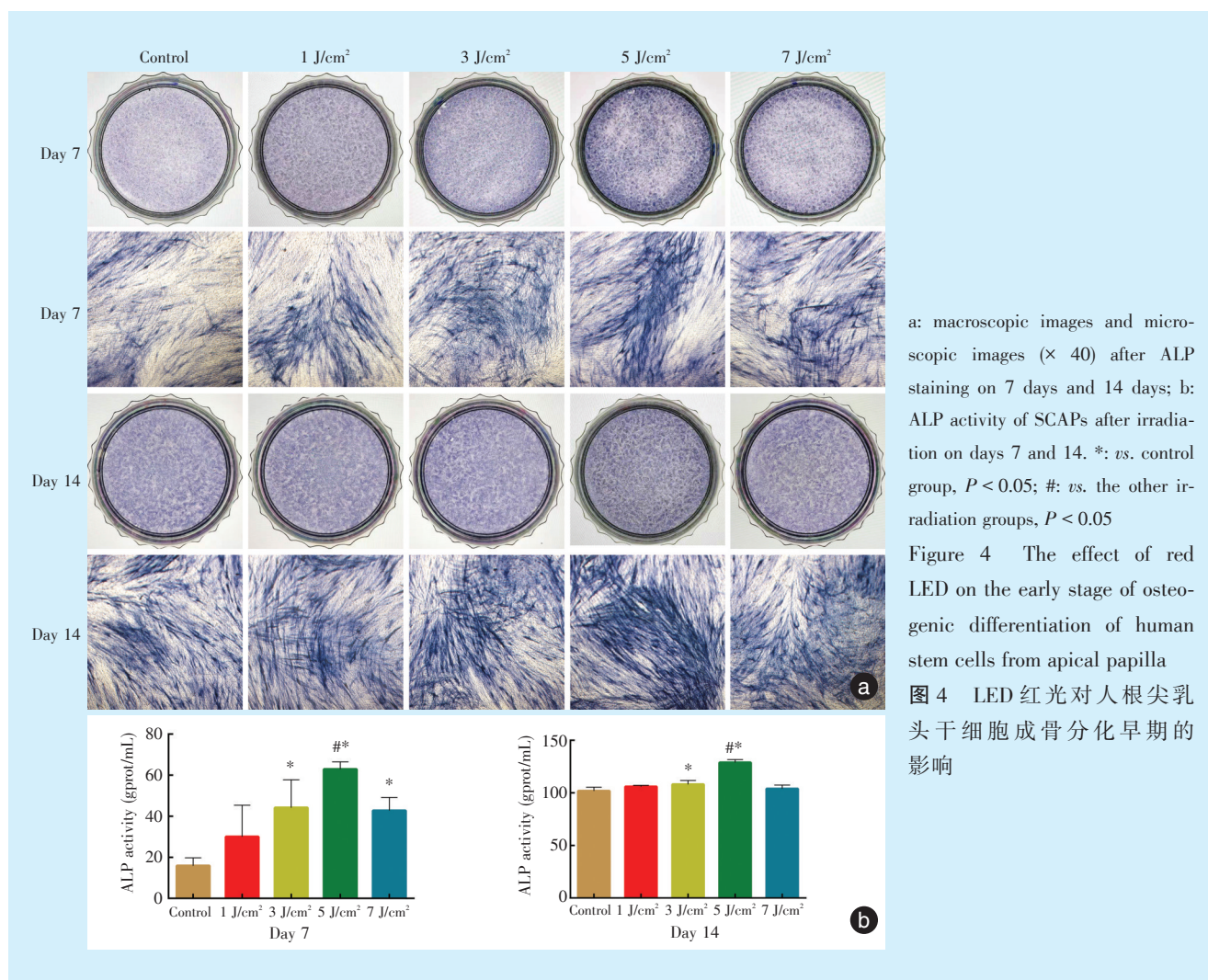
红光照射后,各光照组矿化结节表达量分别相当于对照组的104%、105%、118%和102%,见图5b。

### 2.5 RT-PCR检测LED红光对hSCAPs成骨相关基因表达水平的影响

在光照后第7天,5 J/cm<sup>2</sup>组较对照组上调hSCAPs中成骨相关基因ALP( $F = 784.697, P < 0.001$ ),OCN( $F = 3563.798, P < 0.001$ ),OPN( $F = 511.775, P < 0.001$ ),BSP( $F = 150.117, P < 0.001$ )和Runx2( $F = 713.872, P < 0.001$ )的表达,差异有统计学意义。光照后第14天,5 J/cm<sup>2</sup>组上调hSCAPs中成骨相关基因ALP( $F = 601.905, P < 0.001$ ),OCN( $F = 779.152, P < 0.001$ ),OPN( $F = 1120.033, P < 0.001$ ),BSP( $F = 1736.171, P < 0.001$ )和Runx2( $F = 206.170, P < 0.001$ )的表达,差异有统计学意义,见图6。

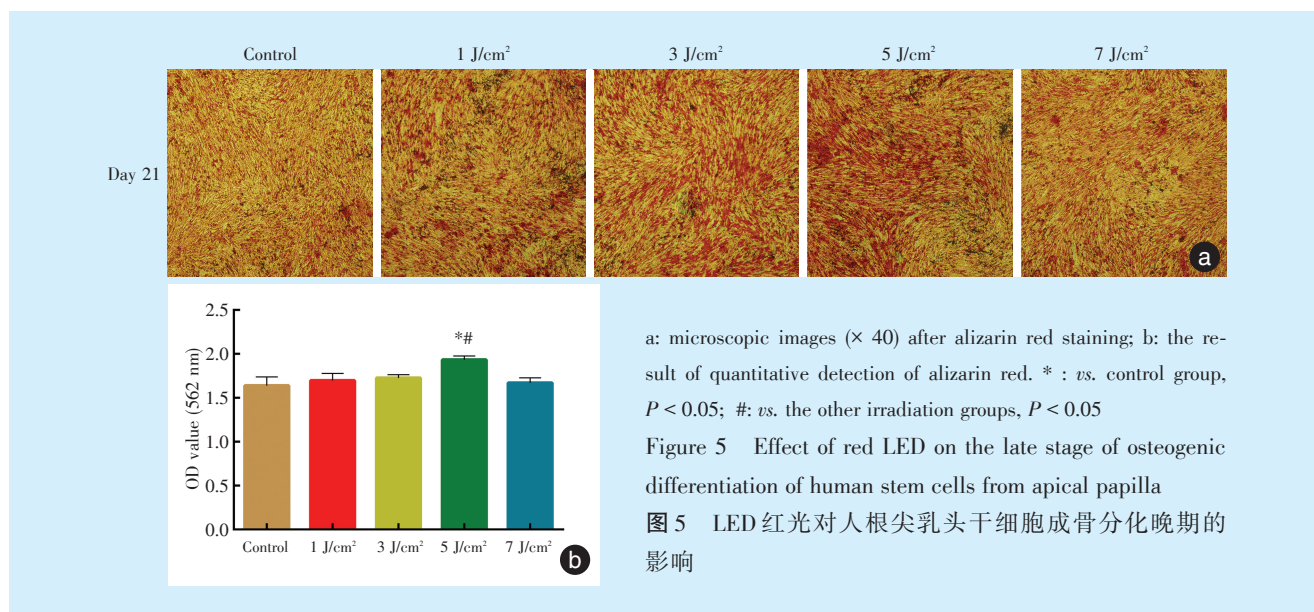
### 2.6 Western blot检测LED红光对hSCAPs成骨相关蛋白表达水平的影响

Western blot结果显示,5 J/cm<sup>2</sup>组在第7天ALP( $F = 172.997, P < 0.001$ ),OCN( $F = 465.951, P < 0.001$ ),OPN( $F = 207.232, P < 0.001$ ),BSP( $F = 209.934, P < 0.001$ )和Runx2( $F = 585.258, P < 0.001$ )蛋白的表达上调,差异具有统计学意义。5 J/cm<sup>2</sup>组在第14天上调ALP( $F = 110.244, P < 0.001$ ),OCN( $F = 182.052, P < 0.001$ ),OPN( $F = 38.148, P = 0.003$ ),BSP( $F = 65.461, P = 0.001$ )和Runx2( $F = 79.046, P = 0.001$ )蛋白的表达,差异具有统计学意义,见图7。



a: macroscopic images and microscopic images ( $\times 40$ ) after ALP staining on 7 days and 14 days; b: ALP activity of SCAPs after irradiation on days 7 and 14. \*: vs. control group,  $P < 0.05$ ; #: vs. the other irradiation groups,  $P < 0.05$

Figure 4 The effect of red LED on the early stage of osteogenic differentiation of human stem cells from apical papilla  
图4 LED红光对人根尖乳头干细胞成骨分化早期的影响



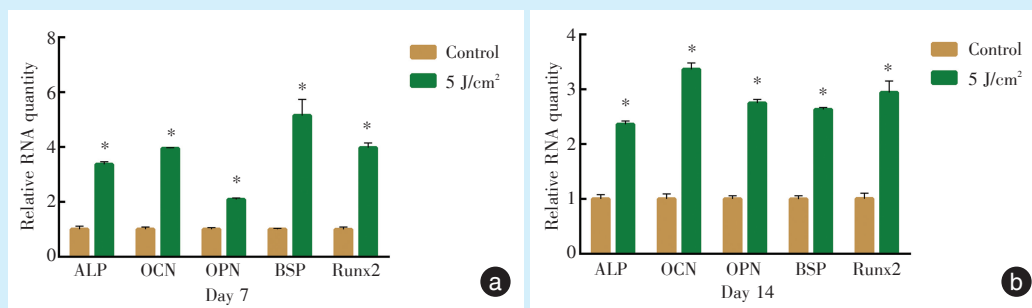
a: microscopic images ( $\times 40$ ) after alizarin red staining; b: the result of quantitative detection of alizarin red. \*: vs. control group,  $P < 0.05$ ; #: vs. the other irradiation groups,  $P < 0.05$

Figure 5 Effect of red LED on the late stage of osteogenic differentiation of human stem cells from apical papilla  
图5 LED红光对人根尖乳头干细胞成骨分化晚期的影响

### 3 讨论

间充质干细胞因其强大的自我更新和分化能

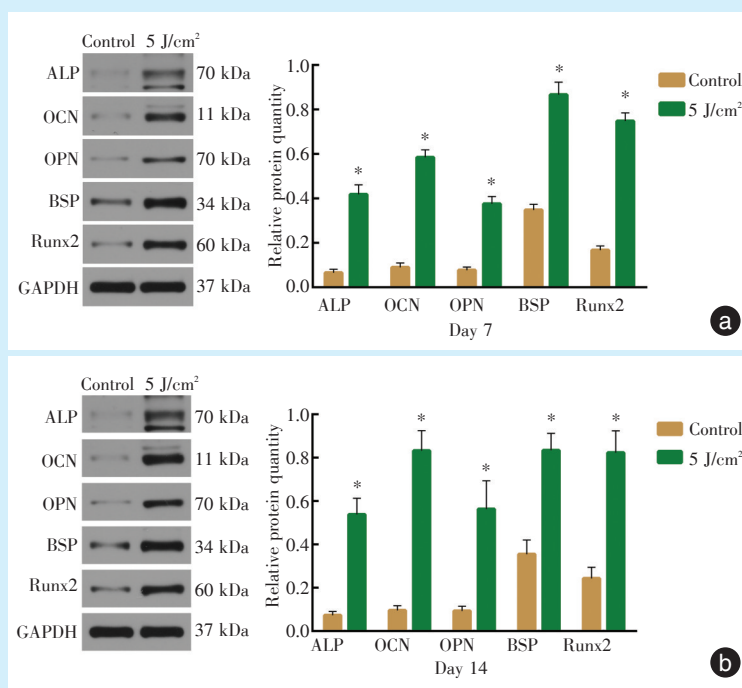
力,成为再生疗法中日益重要的细胞来源<sup>[11]</sup>。hSCAPs具有类似于神经嵴细胞的增殖和分化特征,



a: the expression of osteogenic related genes of hSCAPs on day 7; b: the expression of osteogenic related genes of hSCAPs on day 14. ALP: alkaline phosphatase; OCN: osteocalcin; OPN: osteopontin; BSP: bone sialoprotein; Runx2: Runt-related transcription factor 2; \*: vs. control group,  $P < 0.05$

Figure 6 Effect of red LED on the expression of osteogenic related genes of human stem cells from apical papilla

图6 LED红光对人根尖乳头干细胞成骨相关基因表达的影响



a: the result of electrophoretic bands and expression of osteogenic related proteins of hSCAPs on day 7; b: the result of electrophoretic bands and expression of osteogenic related proteins of hSCAPs on day 14. ALP: alkaline phosphatase; OCN: osteocalcin; OPN: osteopontin; BSP: bone sialoprotein; Runx2: Runt-related transcription factor 2; \*: vs. control group,  $P < 0.05$

Figure 7 Effect of red LED on the expression of osteogenic related proteins of human stem cells from apical papilla

图7 LED红光对人根尖乳头干细胞成骨相关蛋白表达的影响

免疫原性低,有助于组织再生和修复<sup>[5]</sup>。年轻恒牙疾病在保守治疗后可能出现牙髓坏死和根尖周病变,但其牙根仍能继续发育,表明hSCAPs可能在牙髓坏死过程中存活,促进牙根的继续发育<sup>[12-13]</sup>。一定条件下hSCAPs可以分化形成牙髓-牙本质复合体<sup>[14]</sup>、牙周组织<sup>[15]</sup>和骨组织<sup>[16]</sup>。因此,hSCAPs不仅具有发育成生物根潜力,促进牙根继续发育,在牙周软硬组织再生中也能发挥作用,可以用于组织工程和干细胞治疗研究。

LED红光已被证明具有多种生物调节作用,如减轻炎症和促进伤口愈合<sup>[17]</sup>,具有价格低廉、使用寿命长、电路实现简单、应用安全等优点,仪器便于携带,可以作为在家中进行治疗的工具<sup>[6]</sup>,可以通过多种方式影响不同干细胞的增殖和成骨分化<sup>[18-19]</sup>。现阶段LED红光对牙源性干细胞增殖和成骨分化的影响逐渐引起关注,但其对hSCAPs生物学特性的影响研究较少。Horvát-Karajz等<sup>[20]</sup>发现细胞经光照后的光生物学效应可以持续

48 h。Marques 等<sup>[21]</sup>发现 1.2 ~ 7.5 J/cm<sup>2</sup> 的红光能对干细胞的细胞存活率和增殖产生积极效应。同时本课题组前期实验发现 1、3、5 J/cm<sup>2</sup> 的 LED 红光对牙源性间充质干细胞有积极作用<sup>[10]</sup>。故本实验选择 1、3、5、7 J/cm<sup>2</sup> LED 红光, 每 48 h 对细胞进行一次照射, 探讨 LED 红光对 hSCAPs 增殖及成骨分化的影响, 以期为光生物疗法在牙组织工程中的应用筛选适宜的光学参数, 为 hSCAPs 作为牙再生种子细胞提供实验数据。

Hamblin 等<sup>[6]</sup>认为光生物调节作用存在双相剂量反应, 即低剂量光照具有促进作用, 高剂量光照产生抑制作用, 可能是细胞色素 C 氧化酶光受体吸收 LED 红光后被激活, 提供更高速率的氢质子泵入线粒体, 产生能量, 并催化产生活性氧 (reactive oxygen species, ROS) 促进细胞增殖。Ferreira 等<sup>[9]</sup>报道 5 J/cm<sup>2</sup> 红光促进牙髓干细胞增殖, Marques 等<sup>[21]</sup>发现 5 J/cm<sup>2</sup> 红光照射乳牙牙髓干细胞的存活率和增殖率较高, Yamauchi 等<sup>[22]</sup>发现 8 J/cm<sup>2</sup> 对 PDLSCs 增殖促进效果最显著。本实验中, 增殖实验结果也显示 LED 红光在 hSCAPs 增殖早中晚期均有不同程度的促进作用, 5 J/cm<sup>2</sup> 红光照射对 hSCAPs 的促进作用最显著, 提示 LED 红光照射对不同干细胞产生的光生物学效应有微小差异。

本实验发现在成骨诱导条件下, LED 红光促进 hSCAPs 的成骨分化及钙结节的形成, 其中 5 J/cm<sup>2</sup> 组在第 7、14 和 21 天均有良好的促进作用, 表明 5 J/cm<sup>2</sup> LED 红光可以在 hSCAPs 早中晚期成骨中均发挥有利作用。根据以上结果, 笔者选择 5 J/cm<sup>2</sup> 组与对照组进行 PCR 和 western blot 实验探讨相关机制。ALP 和 Runx2 是成骨分化早期的标志<sup>[23-24]</sup>, OCN 在成骨细胞分化晚期表达<sup>[25]</sup>。OPN 的表达与骨形成/吸收相关, 是成骨分化中晚期的标志<sup>[26]</sup>。BSP 在骨形成开始时、成骨分化后期和矿化早期表达<sup>[27]</sup>。结果显示 5 J/cm<sup>2</sup> LED 红光能上调 ALP、Runx2、OCN、OPN、BSP 基因和蛋白的表达, 进一步提示 LED 红光促进 SCAPs 成骨分化。Li 等<sup>[28]</sup>报道 LED 红光促进大鼠骨髓间充质干细胞成骨分化, Ruan 等<sup>[18]</sup>发现 LED 红光具有促进人骨髓间充质干细胞成骨分化的作用, 与本研究结果一致。但 Pagan 等<sup>[29]</sup>提出 LED 红光不影响前成骨细胞 MC3T3 细胞的分化, 这可能与 LED 红光照射曝光量等光学参数不一致有关, 也可能与 LED 红光选择性促进干细胞成骨分化有关。

综上所述, 本实验探讨 LED 红光对 hSCAPs 增

殖和成骨分化的量效关系, 发现在模拟成骨诱导环境中, LED 红光促进 hSCAPs 体外增殖和成骨分化, 其中 5 J/cm<sup>2</sup> 的光照效果最显著, 为促进 hSCAPs 和光生物调节疗法在组织工程和干细胞治疗中的应用提供了新的研究基础。

**【Author contributions】** Su YT performed the experiments and wrote the article. Hou L, Jiang B, Zheng GZ, Liu Y and Wang Y performed the experiments and revised the article. All authors read and approved the final manuscript as submitted.

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