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Biostimulation of dermal fibroblast by sublethal Q-switched Nd:YAG 532 nm laser: Collagen remodeling and pigmentation

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Abstract

The application of medical lasers in treating pigmented lesions has rapidly developed over the past decade. In both clinical and cosmetic application, melanin is targeted in pigmented areas and destroyed by the mechanism of selective photothermolysis. When laser radiation passes through superficial pigmented tissue, energy will be further reduced by dermal collagen scattering and absorption. Non-pigmented dermal fibroblasts will be exposed to co-incidental laser irradiation at lower energy levels. Biostimulation of dermal fibroblasts by low energy laser is reported in this paper. The Q-switched frequency doubled Nd:YAG 532 nm laser used in clinical laser therapy was used in this study. Sublethal laser fluence was determined at 0.8 J/cm^2 and used to stimulate normal human fibroblasts in monolayer culture. The results showed that there was no significant difference in collagen synthesis between the stimulated fibroblasts and controls. However, significant delay in collagen remodeling activity was demonstrated in the irradiated group by measuring fibroblast populated collagen lattice (FPCL) contraction. The stimulation of SCF, HGF and b-FGF gene expression was determined by RT-PCR analysis and demonstrated to vary between cases. Two out of six cell lineages that showed stronger responses to laser stimulation on SCF, HGF and b-FGF gene expressions were used to prepare conditioned media. The conditioned media from irradiated groups showed significant increase in SCF and b-FGF content and stimulated SK-mel-3 melanoma cells to synthesize more melanin in vitro. These results suggest that sublethal laser stimulation of fibroblasts may cause post-laser hyperpigmentation through production of melanogenic stimulatory cytokines. The degree of stimulation of SCF, HGF and b-FGF production varied between individual cell lineages, which may reflect the true variation of post-laser hyperpigmentation in clinical practice.

Keywords: Fibroblast; Laser; Pigmentation

1. Introduction

The frequency doubled Nd:YAG laser (532 nm) is widely used in treating pigmented lesions based on the principles of selective photothermolysis first described by Anderson and Goldberg [1,2]. The laser in the Q-switched mode is designed to deliver energy with pulse duration (10^{-9} s) shorter than the thermal relaxation time of melanin (5×10^{-8} s) located in the target cell, thereby localizing the effect of the thermolysis. The target cell is then heated up to 70–100 °C leading to denaturation of protein, nucleic acid and lipid membrane and thus finally undergoes coagulative necrosis. In addition to the photothermal effects of coagulation and vaporization, laser energy can interact with tissue to form photomechanical shock waves [3] or photochemical biostimulations (review in [4]). Karu has suggested that some native cellular components are photoacceptor in the process of biostimulation by photons [5]. She described four possible primary biostimulatory reactions that formed the biophysical

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basis of the evolving low-power laser therapy. In our previous study, sublethal Q-switched 532 nm laser energy stimulation on amelanotic G361 melanoma cell was shown to enhance melanoma specific metastatic marker expressions including integrins α3, α4, β1 and focal adhesion kinase [6]. This suggested that the changes are induced by photochemical biostimulation rather than photothermal effect.

Post-treatment laser hyperpigmentation is one of the complications in Q-switched 532 nm laser therapy. It has been reported to occur in up to 24% of oriental patients receiving treatment for facial lentigines [7]. The regulation of human pigmentation is a complex, multifactorial process involving such factors as genetic inheritance, environmental stimuli, hormonal status, and anatomical site. Many studies illustrate the importance of keratinocytes in the regulation of melanocytes [8,9]. Recent studies on fibroblasts reported a regulatory role in the control of pigmentation; fibroblast-derived stem cell factor (SCF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (b-FGF) can stimulate growth and melanogenesis of melanocytes in a monolayer coculture system [10]. Fibroblast-derived extracellular matrix also influences melanocyte orientation in the epidermis within a three-dimensional skin model [11,12]. In laser therapy, laser fluence is usually set to achieve a treatment endpoint denoted by immediate whitening of the epidermis without bleeding [7]. The intensity of laser energy decreases with increasing depth in the skin tissue by absorption and scattering in accordance with the Beer–Lambert exponential law [13]. The epidermal keratinocytes above the pigmented lesion are subject to vaporization and coagulation by temperature elevated above 60 °C and the dermal fibroblast underneath is exposed to sublethal energy laser. We hypothesize that the dermal fibroblasts are biostimulated by sublethal laser energy and may thus play a role in hyperpigmentation through paracrine action. In this study we initially established a dose response curve of human fibroblasts derived from normal skin to the Nd:YAG 532 nm laser and investigated changes in cell proliferation, collagen synthesis and collagen remodeling activity after exposure to the laser. We further looked at the stimulation of melanogenesis stimulatory cytokine production and the action of fibroblast-conditioned medium on melanogenesis using a moderate melanin-containing melanoma cell line (SK-mel-3).

2. Materials and methods

2.1. Cell culture

Normal human fibroblast culture was established by explant techniques using foreskin or normal skin tissue discarded during surgical procedures following our institutional ethical guidelines. Briefly, skin specimens were collected in Dulbecco's modified eagle medium (DMEM) + 10% fetal bovine serum (FBS) supplemented with anti-biotics and anti-mycotics solution (containing 100 U/ml penicillin, 100 μg/ml streptomycin and 250 ng/ml fungizone®) and 50 μg/ml gentamicin. The skin surface was then decontaminated by rinsing in 70% ethanol for 1 min, followed by three washes with phosphate buffered saline (PBS). The tissue was then minced by scalpel and scissors to 1–2 mm cubes and evenly distributed on tissue culture plates. The tissue was incubated for 2 h in an up-side-down position at 37 °C, 5% CO_2, to facilitate better attachment. DMEM supplemented with 50% FBS was used in the first week of culture and medium not changed. Subsequently, DMEM + 20% FBS was used and medium was changed twice per week until outgrowth of fibroblasts observed. Fibroblasts were subcultured by trypsinization once 90% confluence was reached and expanded in 1:5 ratio in DMEM + 10% FBS. All fibroblasts used in this study were from passage 5–8. Culture reagents were purchased from Gibco-BRL (Life Tech., CA, USA) and culture wares were from Nunc (Rochester, NY).

Melanoma cell line SK-mel-3 was purchased from ATCC (Rockville, MD) and maintained in DMEM + 10% FBS with medium changed twice per week.

2.2. Laser specification

Q-switched frequency doubled Nd:YAG laser at 532 nm, with pulse width 4 ns, was generated from Versapulse® C Cosmetic Laser System (Coherent medical group) and delivered by handpiece with adjustable spot sizes of 2, 3, 4, 5 and 6 mm in diameter. This system has maximum energy of 200 mJ per pulse and treatment repetition rate as single shot, 1, 2, 5 and 10 Hz.

2.3. Cell viability

The dose–response curve of normal human skin fibroblasts was established by trypan blue exclusion assay using two fibroblast lineages. Fibroblast cultures were set up using 6-well plates by seeding 5×10^4 cells/well in DMEM + 10% FBS. After culturing for 2 days, upon all wells reached 90% confluence, medium was withdrawn and the wells were rinsed once with PBS. Triplicate wells were treated with laser at fluences of 0, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, and 4.0 J/cm^2 by placing the culture plate in an inverted position to make the culture surface close to the end of the handpiece. Single exposure of laser to culture surface was achieved by aligning a transparent grid placed on top of the flask and the burn mark generated on a red cardboard backing placed underneath the culture plate. DMEM + 10% FBS was replenished to each well after