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RESEARCH ARTICLE

The impact of the particle size of curcumin nanocarriers and the ethanol on beta_1-integrin overexpression in fibroblasts: A regenerative pharmaceutical approach in skin repair and anti-aging formulations

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Abstract

Background Since women pay more attention to their skin's health, pharmaceutical companies invest heavily on skin care product development. Further, the success of drug nano-carriers in passing through the skin justifies the need to conduct studies at the nano-scale. β1-integrin down regulation has been proposed as a sign of skin aging.

Methods Six drug nano-carriers (50 and 75 nm) were prepared at three ethanol concentrations (0, 3,and 5%) and different temperatures. Then, the impact of Nanocarriers on fibroblasts were investigated.

Results DLS showed that increasing ethanol concentration decreased the surface tension that caused a decrease in the particle size in non-temperature formulations while increasing the temperature to 60 °C to lower Gibbs free energy increased the particle size. Ethanol addition decreased β1-integrin over-expression, whereas larger nano-carriers induced an over-expression of β1-integrin, Bcl2/Bax ratio, and an increase in live cell number. β1-integrin over-expression did not correlate with the rate of fibroblast proliferation and NFκB expression. An increase in fibroblast mortality in relation to smaller nano-carriers was not only due to the increase in Bax ratio, but was related to NFκB over-expression.

Conclusion The development of a regenerative pharmaceutical approach in skin repair was based on the effect of particle size and ethanol concentration of the drug nano-carriers on the expression of β1-integrin in fibroblasts. A curcumin nanoformulation sized 77 nm and containing of 3% ethanol was more effective in increasing β1-integrin gene over-expression, anti-apoptosis of fibroblast cells (Bcl2/Bax ratio), and in decreasing Bax and NFκB gene expression than that with a particle size of 50 nm. Such a formulation may be considered a valuable candidate in anti-aging and wound-healing formulations.

Keywords Particle size · Nanocarriers · Surface tension, chemical stability, integrin β1 · Skin aging · Wound healing.

Background

Nowadays, females have more concerns about skin health than ever before. This point encourages the pharmaceutical companies to invest on cosmetic industries. To achieve this goal, it is necessary to have adequate and accurate information about pharmaceutical dermal products. Employment of drug nanocarriers seems to be a proper strategy to effectively release and penetrate drug combinations into the skin.

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The impact of nanotechnology in tissue engineering has been led to desinging of more smart and bio-similar scaffolds. There have diversity at the level of particle or fiber size and also compartments [1, 2]. Whether particle size (50–80 nm) and ethanol concentration (0%–5%; ethanol as a cosurfactant) influence the expression of integrin-ß1 (a cell surface receptor) and NFκB (an inflammatory factor) is an unanswered question in dermal drug delivery. Dermal fibroblasts, as major mesenchymal cells in the dermis, contribute to skin integrity and structure. They are responsible for collagen synthesis, while playing a critical role in skin aging and rejuvenation [3].

It is noteworthy that the Food and Drug Administration (FDA) approved dermal fibroblasts for clinical applications [4]. Dermal fibroblasts are involved in four main phases of wound healing including hemostasis, inflammation, proliferation, and remodeling. Dermal fibroblasts trigger the inflammatory phase through secretion of extracellular matrix (ECM) biomolecules and then proliferate and differentiate into myofibroblasts to induce wound contracture. In the final phase, myofibroblasts undergo remodeling and apoptosis, while the remaining dermal fibroblasts secrete type I collagen [4].

Due to some barriers such as the stratum corneum of the skin, drug delivery to this site can be challenging, resulting in decreased transdermal drug efficacy. Application of a pharmaceutical agent with proper physicochemical properties such as unsaturated fatty acids and nanometer particle size induces synergistic effects to increase drug penetration into the skin [5]. Therefore, application of drug nanocarriers, which can cross the stratum corneum and reach the dermis more efficiently, is of interest to researchers [5].

The curcumin, as the bioactive compound of turmeric, is an anti-inflammatory and antioxidant agent decreasing aging through NFκB inhibition [6], lipofuscin reduction, and superoxide increment [7–9]. It seems that encapsulation into a nanocarrier increases the efficacy of this extract and induces its synergistic effects. Therefore, in the current study, curcumin was encapsulated in a nanocarrier at two different particle sizes, and ß1-integrin gene overexpression was compared.

First, cells respond to the environment via cell surface receptors such as ß1-integrin, while this interaction is disrupted in the absence of such receptors. In this regard, Giangreco et al., indicated that the level of ß1-integrin was significantly higher in the skin of younger donors than the older ones. There is a direct relationship between aging and integrin-ß1 downregulation, while ß1-integrin increment is significantly associated with skin self-renewal [10]. However, other researchers indicated the role of ß1-integrin in epidermal proliferation, basement membrane formation, and hair follicle morphogenesis in the skin of mice [11].

Liu et al., reported that ß1-integrin ablation in fibroblasts results in delayed wound healing, reduces the production of new ECM and type I collagen, decreases granulation tissue formation, and inhibits fibroblast differentiation into myofibroblasts [12]. Besides, Rozo et al., indicated the role of ß1-integrin in the self-renewal of satellite cells through restoring fibroblast growth factor (FGF-2) sensitivity [13].

To the best of authors` knowledge, no study investigated the effect of particle size on ß1-integrin gene expression so far. Therefore, evaluation of the effects of particle size on ß1-integrin expression in fibroblasts can be useful to design pharmaceutical agents to recover aging. The current study aimed at investigating the impact of particle size and ethanol concentration in curcumin nanocarriers on the overexpression of ß1-integrins as cell surface receptors at the mRNA level.

NFκB is considered as a critical mediator of aging [14] activated by oxidative stress and inflammatory agents. On the other hand, it activates inflammatory cells involved in the process of aging, as well. NFκB acts both as a prosurvival and cell apoptotic factor in different cells [15]. Kriete et al., revealed that NFκB and inflammatory factors are more activated in the dermal fibroblasts of the elderly people than the younger ones [16]. However, NFκB reduction in the skin of old mice led to the improvement of age-related pathologies [14].

With regard to the importance of fibroblasts in wound healing and skin repair and the role of ß1-integrin in aging, Curcumin nanocarriers was prepared with six formulations, two particle sizes (50 and 70 nm), and three ethanol concentrations (0%, 3%, and 5%) to evaluate particle size and ethanol concentration of nanocarriers effects on ß1-integrin and NF B gene expression, cell proliferation, and fibroblast viability.

Methods

Standard curve of curcumin

The maximum wavelength of curcumin was determined using Spectroscopic analysis and then its standard curve was drawn. In brief, a stock solution of curcumin containing 3.5 mg/ml was prepared in ethanol and then, its standard serial dilution was made. The absorbance of the sample was measured at the maximum absorbance using UV-Vis spectrophotometer. The assay was performed in triplicate and values provided are the mean ± SD of three independent experiments.

Screening of oil

The solubility of curcumin in mineral, sesame and soybean oils was determined by adding 1 mg of curcumin in 1 ml of oils separately using a spectrophotometer. Then, they were stirred (100 RPM) at 25 ± 1.0 °C for 48 h to achieve equilibrium. After 48 h, samples were centrifuged at 3000 RPM for 15 min and 100 μl of supernatant was taken and diluted with solvent. The absorption was triplicate measured using a UV-Vis spectrophotometer at 425 nm.

Nano- carrier preparation

Curcumin nanocarriers were prepared using a low energy (oil in water) emulsification method. Briefly, curcumin was added to the soybean oil at the final concentration of 3%. Then to obtain HLB 7, they were mixed with both non-ionic surfactants of Tween and Span 80 at the final concentration of 10%. Tween 80 is a polyoxyethylene Sorbitan monooleate emulsifier (hydrophil) while Span 80 is a sorbitan monooleate emulsifier (lipophil). Ethanol as a co-surfactant was added at the final concentrations of 0 and 3%, as well. After the homogeneously stirring of the mixture for 5 min, water drop-wise was added at low (25 °C) and high (60 °C) temperatures at 1200 RPM under stirring condition. The samples with poor ethanol were named 3.10 (25 °C) and 3. 10 T (60 °C) while nanocarriers with 3% ethanol were named 3.10.3 (25 °C) and 3.10.3T (60 °C).

Nano-carriers characterization

Hydrodynamic particle size

It was prepared six different types of curcumin nanocarriers and they were denoted as 3.10, 3. 10 T, 3.10.3, 3.10.3T, 3.10.5 and 3.10.5T with the characteristics presented in Table 1. The hydrodynamic particle size of 3.10, 3. 10 T, 3.10.3, 3.10.3T, 3.10.5 and 3.10.5T were measured using a laser light scattering technique (Qudix scateroscope I (Korea)) with refractive index of 1.42 [17]. To prepare an inert medium for double layer of nano carriers, they were disperesed in 10 mM monovalent electrolyte [18]. Light scattering was monitored by 300 s and the hydrodynamic size of nanocarriers were measured in intensity mode.

Thermodynamic stability testing of curcumin nanocarriers

Thermodynamic stability of nanoemulsions was performed as following. The first step was two freeze- thaw cycles between −20 °C (24 h) and room temperature (25 °C). Then, it was followed by the centrifugation at 5000 RPM for 30 min. The final step was the six cycles of heating (40 °C) and cooling (4 °C) by 48 h. The stable nanoemulsions do not show phase separation or turbidity in each steps [19].

Chemical structure, stability testing of curcumin nanocarriers by TLC

To investigate whether the formulations (surfactant and co-surfactant) and temperatures degrade or damage to the chemical structure of curcumin, thin layer chromatography was performed. However, to compare the efficacy of two recommended mobile phases, stationary phase separately kept in mobile phases. Briefly, 10 μl of nanoemulsions were spotted in 1 cm above the margin of silica gel F254 aluminum plate (stationary phase). The plates were separately immersed in two glass chambers containing hexane: ethyl acetate [7:3] and chloroform: methanol [1:20] as a mobile phase. Retention factor (Rf), is the distance migrated over the total distance covered by the solvent. Then, bonds were visualized and captured under UV irradiation equipped with a camera [20]. The analysis was repeated 3 times.

pH measurement

The apparent pH of curcumin nanocarriers was measured using a pH meter (Crison, Medidor PH BASIC 20, Spanish) in triplicate at 25 °C.

Entrapment efficacy by ultrafiltration method

The unloaded curcumin was determined using separation by ultrafiltration method. In brief, 500 μl of nano-formulations was added to Amicon ultra-0.5 ml (molecular weight cutoff 10 kDa). Microtubes were centrifuged for 15 min at 14000 G. The unloaded curcumin was collected and absorbance was read using spectrophotometer at 425 nm. The concentration of curcumin was determined using the formula derived from curcumin standard curve. The assay was performed in triplicate and values provided are the mean ± SD of three independent experiments.

Table 1 Curcumin Nanocarriers characterization

Name	Temperature	Ethanol concentration	Particle size (nm)	pH	Chemical stability	Thermodynamic stability	EE (%)
3.10	25	0	56.8 ± 0.1	5.2 ± 0.3	Stable	Stable	99.9 ± 0.3
3. 10 T	60	0	78.3 ± 0.85	5 ± 0.2	Stable	Stable	99.4 ± 0.6
3.10.3	25	3	56.8 ± 0.1	5.3 ± 0.3	Stable	Stable	99.65 ± 0.7
3.10.3T	60	3	78.3 ± 0.85	5.2 ± 0.5	Stable	Stable	98.3 ± 1.1
3.10.5	25	5	47.9 ± 1	5.2 ± 0.2	Stable	Stable	99.35 ± 0.8
3.10.5T	60	5	70.7 ± 1	5.3 ± 0.1	Stable	Stable	99.05 ± 0.41

Cell metabolic activity using MTT assay

The cell metabolic activity evaluation of nanocarriers was performed using MTT assay on L929 cells as a Mouse fibroblast cell line (Pasteur, Iran (C161)). 5×10^3 cells/well at passage three were seeded triplicate in 96-well plate containing DMEM as a cell culture medium supplemented with 10% FBS (GIBCO), 1% Penicillin/Streptomycin (BIO-IDEA, Iran) for 24 h. They were incubated at 37 °C in a 5% CO_2 incubator. Then, the media was completely exchanged with fresh medium containing drug nanocarriers at the final concentration of 312.5 ng/ml for 48 h at 37 °C in a 5% CO_2 and 95% moisture incubator. Afterwards, the media were completely removed and exchanged with the 100 μl of 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium (Sigma, USA) (0.5 mg/ml PBS) for 4 h. Then, dimethyl sulfoxide (DMSO; Sigma, USA) was added to dissolve hydrazine crystal and the plate was read in a microplate reader (BioTek) at 570 nm up to 20 min. The assay was performed in triplicate and values provided are the normalized mean ± SD of three independent experiments.

Cell viability assessment using PI flow cytometry

Propidium iodide as a marker of cell death, both late apoptotic and necrotic cells was used for cell viability investigation. After the cell seeding (3×10^4 and passage three) in a 24-well plate, the cells were treated with 312.5 ng/ml of drug nano-carriers at 37 °C in a 5% CO_2 and 95% moisture for 48 h. To quantitatively investigate dead cells, flow-cytometry was performed. Following the cell trypsinization, they were centrifuged at 1200 RPM for 5 min and washed with PBS. PI solution was added and incubated at dark room temperature and flow cytometry was performed up to 30 min. The assay was performed in triplicate and values provided are the normalized mean ± SD of three independent experiments.

β1-integrin, NFκB, Bcl2/Bax ratio genes expression

To quantify the relative fold change gene expression at the level of mRNA for β1-integrin, NFκB and Bcl2/Bax ratio, L929 cells (1×10^5 cell/well) in passage three were seeded in a 6-well plate supplemented with RPMI, 10% FBS, 1% penicillin/streptomycin overnight. Then, the media was completely exchanged with fresh media containing 312.5 ng/ml of nano carriers by 48 h. RNA was extracted from treated cells using RNX- Plus kit (Sina clon, Iran) and treated with DNAse I. cDNA (1 μg) was synthesized using a TAKARA cDNA synthesis kit (Burlington, Japan). cDNA was used for 45 cycles PCR in Rotor-gene Q real time analyzer (Corbett, Australia) using Eva Green master mix. Each reaction was performed in duplicate and values provided are the normalized mean ± SD of three independent experiments. The relative fold change gene expression was quantified using the DDCt method. The primer sequence of genes is listed in Table 1.

Results

Oil screening of curcumin

Curcumin was dissolved in different oils and results showed that there were no significant differences between the solubility of curcumin in soybean oil, sesame oil and mineral oil ($P > 0.05$). However, sesame oil and soybean oil significantly dissolved curcumin than mineral oil ($p < 0.01$ and 0.001, respectively) (Fig. 1a).

To evaluate EE% and drug release, curve standard of curcumin is necessary and results showed a linear relationship between concentration and absorbtion at 425 nm (Fig. 1b).

Temperature exhibited significantly larger particle size of nanocarriers

3.10, 3.10.3, 3.10.5, 3. 10 T, 3.10.3T, 3.10.5T nanocarriers were prepared by the non-energetic method of stirring at 22 and 60 °C, respectively. Results showed that temperature induced larger particle size of 78.3 ± 0.85 nm in 3. 10 T and 3.10.3T nanocarries as compared to the nanocarriers of 3.10 and 3.10.3 (56.8 ± 0.1 nm). Although 3.10.5 and 3.10.5T were compliant with the mentioned trend, the hydrodynamic particle size of them were less than other non-temperature and temperature formulations (47.9 ± 1 and 70.7 ± 1, respectively). It is worthy of value to be mentioned that the poly disparity index (PDI) of all nanoformulation was less than 0.3 and temperature nano-formulations exhibited non- significant higher PDI value (Table. 1).

Besides, pH measurement data disclosed that there were no significant differences between all nano-formulations and they were in the range of 5.2 ± 0.12 ($p > 0.05$).

Temperature and ethanol concentration do not degrade the chemical structure of curcumin in nanocarriers and also do not influence on the thermodynamic stability of nanocarriers

TLC was performed to investigate whether temperature and formulation ingredients affected the chemical structure of curcumin. Results showed that not only formulation and temperature did not degrade curcumin but also all nano-formulations had equal R_f of 0.14 same as non-formulated curcumin as a control (Fig. 2).

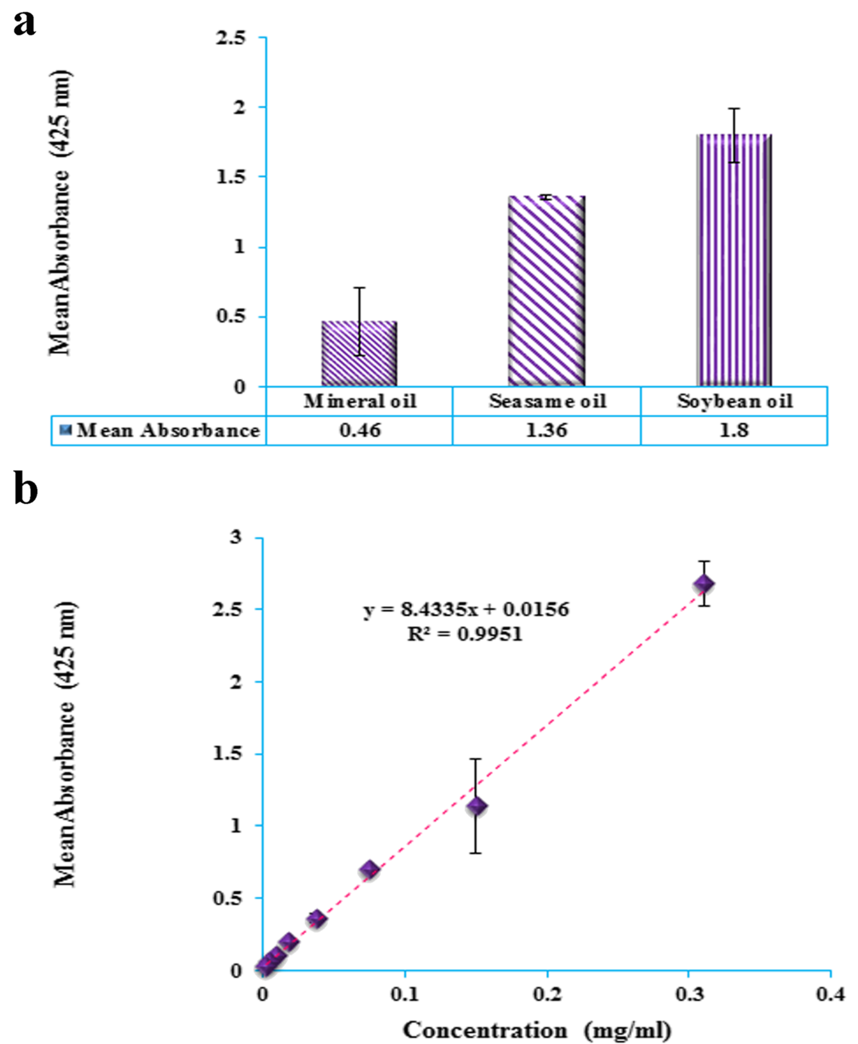

Fig. 1 TLC of curcumin nanocrriers at different concentrations using two mixtures

Thermodynamic stability

Based on thermodynamic stability analysis (freeze-thaw, centrifugation, heating-cooling), there were no sign of change in the appearance and biphasic state of nano-formulations and all nanoformulations showed thermodynamic stability (Table. 1).

EE% measurements

The percentage of EE non-significantly decreased in non-temperature or smaller (~50 nm) nanoformulations with ethanol increment from 99.9 to 99.35%. Besides, larger nanoformulation showed non-significantly less EE% than smaller ones (Table 1).

Size and ethanol concentration of nanocarriers influence cell surface receptor of β1-integrin

β1-integrin a cell surface receptor involved in fibrogenesis and skin repair was investigated. Results showed that larger nanoparticels induced significantly higher β1-integrin gene overexpression compared to the smaller ones ($p < 0.05$). Besides, increase of ethanol in the larger nanocarriers induced higher β1-integrin gene overexpression. Although this trend continued in smaller nanocarriers, nanocarriers with 3% ethanol concentration induced less β1-integrin gene expression than others ($p < 0.05$) (Fig. 3a).

Bcl2/Bax ratio as an anti-apoptotic marker was in good agreement with β1-integrin gene expression

We were going to curious about the influence of the particle size and ethanol concentration on Bcl/Bax ratio as the index of anti-apoptosis. Larger nano carriers except 3.10.5T lead to an increase in the Bcl2/Bax ratio than smaller ones. Although 3. 10 T and 3.10.3T induced higher Bcl2/Bax ratio than the control group ($p < 0.05$ and 0.001), 3.10.5T induced significantly less Bcl2/Bax ratio than the control group ($p < 0/05$) and there was no significant difference between cell viability of 3.10.5 nanoformulation with the control group ($p > 0.05$), (Fig. 3b).

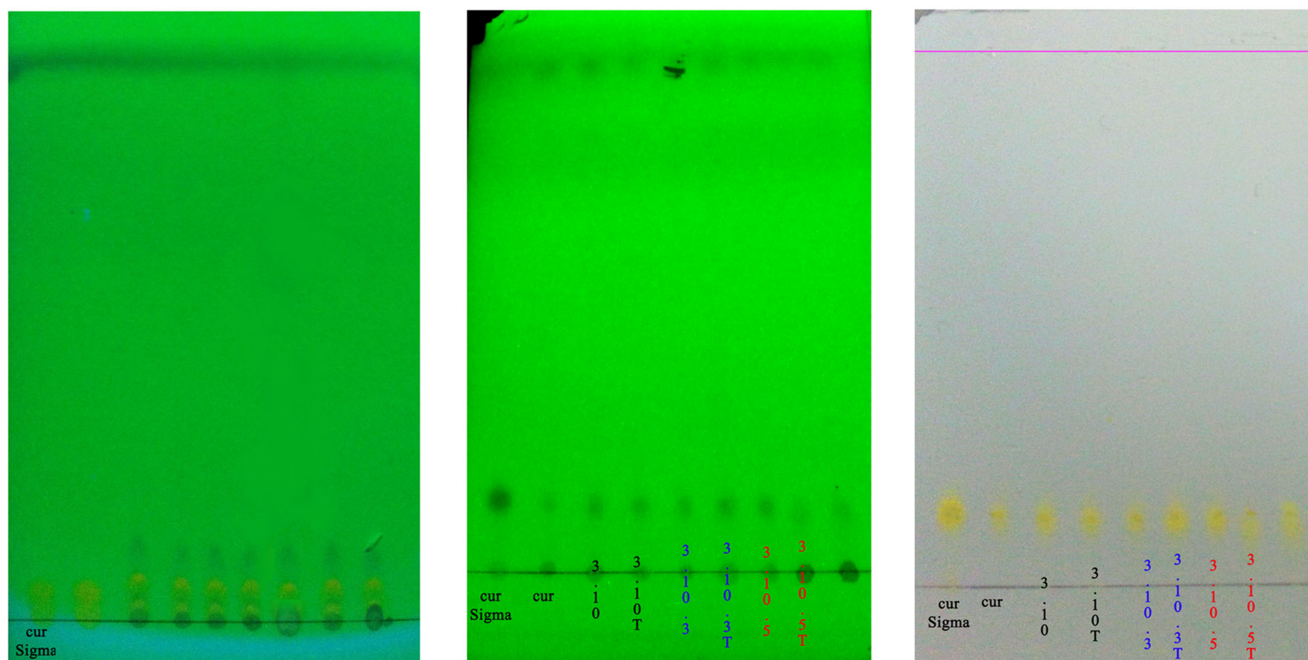

Fig. 2 **a** Oil screening of curcumin with the mineral oil, sesame oil and soybean oil. **b** Curve standard of curcumin solved in ethanol (3mg/ml)

Stimulation of integrin β1 by the size of nanocarriers is opposite of NFκB expression while NFκB expression is in agreement with cell proliferation assay

NFκB as a pro-inflammatory gene involved in ageing and skin repair, was studied. Results showed that smaller nanocarriers induced significantly higher level of NFκB gene expression than larger ones ($p < 0.05$) while, addition of ethanol at both 3 and 5% concentrations decreased the level of NFκB as a pro-inflammatory gene as compared to 0% concentration. Interestingly, there were no significant differences between NFκB gene expression of 3 and 5% ethanol concentrations ($p > 0.05$), (Fig. 3c).

Cell proliferation assay by MTT assay was in opposite of β1-integrin gene expression

Based on MTT assay data, curcumin nanocarriers induced significantly higher cell proliferation than the control group ($p < 0.001$) while the increase of particle size (temperature formulation) and ethanol concentration from 0 to 3% induced significantly less cell proliferation ($P < 0.001$). When size falled into 49 and 70 nm, the trend changed and there was no significant difference between cell metabolic activity of 3.10.5 and 3.10.5T ($p > 0.05$). In other word, both of them induced significantly higher cell proliferation than 3 ($p < 0.05$) and 3 T ($p < 0.01$) respectively. Besides, there were no significant differences between the cell proliferation of 3.10.5T with 3. 10 T and 3.10.3 ($p > 0.05$), and also between 3.10.5T and 3.10.3 ($p > 0.05$). In another word, the increase of ethanol in nano-formulations with the same size ((56.8 nm) and (77.7–78.9 nm)) resulted in less cell proliferation ($p < 0.001$) while in higher ethanol concentration (5%) with smaller particle size (70.7 nm as compared to 77.7 and 78.9 nm), cell proliferation was singnificantly higher than the lesser ethanol concentration (3%) (Fig. 4a).

Smaller nanocarriers induced higher percentage of PI negative cells in agreement with MTT assay data

To confirm MTT assay data, PI flow-cytometry was performed. It was disclosed that larger nanoformulation except 5% ethanol concentration induced significantly less PI positive cells as compared to the smaller nanocarriers. However, the increase of ethanol concentration in small nanocarriers from 0 to 5% did not change dead cells. The percentage of dead cells increased in groups treated with larger nanocarriers around 70 nm at the concentration of 0 to 3%, while it significantly decreased at 5% ethanol concentration ($P < 0.5$), (Fig. 4b).

Discussion

There are diverse strategies to manipulate wrinkle, skin scars and burns using nanofiberous scaffolds [21] and other biomaterials [22]. Meanwhile, there are different reports on the effect

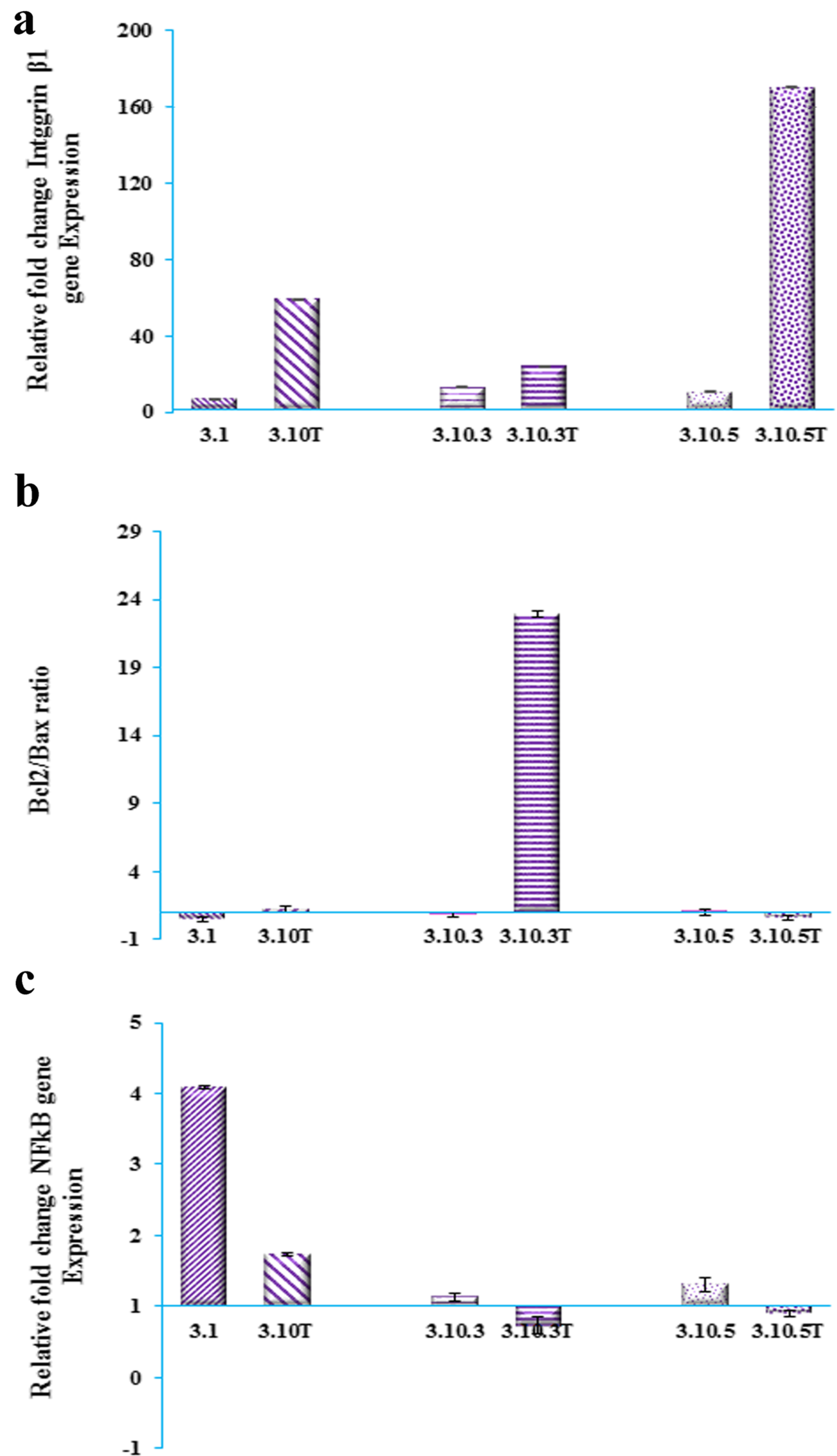

Fig. 3 **a** Integrin β1 gene expression in fibroblasts treated with curcumin nanocarriers. Larger nanocarriers induced significantly higher Integrin β1 gene expression than smaller compartments. **b** NFκB gene expression in fibroblasts treated with nanocarriers. qRT-PCR results showed that larger nanocarriers decreased significantly less level of NFκB gene expression. **c** Bcl2/ Bax ratio in fibroblasts treated with nanocarriers. qRT-PCR results showed that larger nanocarriers increased significantly Bcl2/ Bax ratio as an anti-apoptotic marker

of particle size of nanoparticles [23–25] and nanofiber diameter [26] on bone and nerve regeneration, but there are no reports on the impact of particle size of drug nanocarriers and ethanol concentration on skin repair. Therefore, In the current study, curcumin nanocarriers were prepared with two particle sizes (about 50 and 70 nm), and the effects of temperature and ethanol concentration on particle size, pH, and chemical and thermodynamic stability were studied. Based on the current study findings, temperature increased the particle size of nanocarriers, while there was no significant association between EE% (data not shown) and pH of nanocarriers. Moreover, the released profile of nanocarriers was influenced by ethanol concentration, which increased from 0% to 5% (data not shown). Changes in ethanol concentration (0%–5%) and temperature (60 °C) did not influence the chemical structure or thermodynamic stability of nanocarriers, while

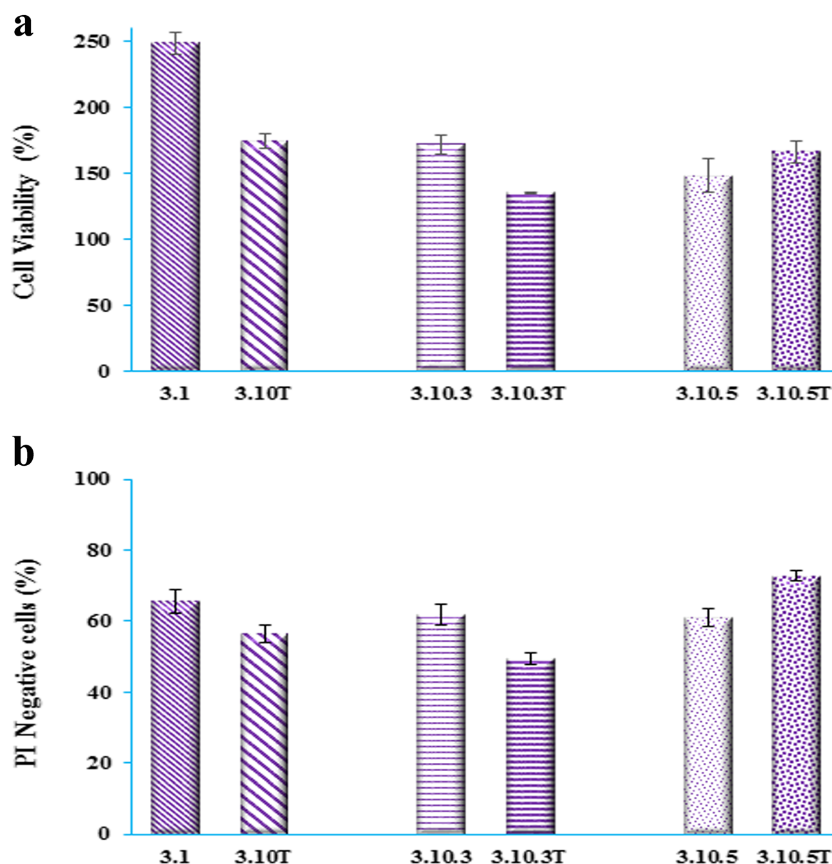

Fig. 4 **a** Cell proliferation of fibroblasts treated with nanocarriers by MTT assay. Smaller nanocarriers induced significantly higher cell proliferation than larger ones. **b** Cell viability of fibroblasts treated with nanocarriers by PI flow-cytometery method. Smaller nanocarriers induced significantly higher cell viability than larger ones with an increase in PI negative cells

hydrodynamic particle size decreased as the ethanol concentration increased from 3% to 5%.

Chin et al. [27] demonstrated that the particle size of starch nanoparticles, prepared by the microemulsion method, decreased relative to cyclohexane/ethanol concentration, which was in good agreement with the current study findings. It can be said that an increase in the level of ethanol as a cosurfactant decreases the interfacial tension of oil and water, resulting in the reduction of particle size. However, an increase in ethanol concentration from 0% to 3% was not sufficient to cover the entire surface of droplet and did not change the particle size; on the other hand, when ethanol concentration increased to 5%, it could cover the entire surface and produced smaller nanoparticles.

Nonetheless, Chin et al. [27] showed that increased ethanol concentration led to an increase in surface fluidity, which in turn improved the intermicellar exchange kinetics; this phenomenon contributes to the homogenous distribution of nanoparticles. Ethanol decreases the particle size in nanoemulsion systems, and its addition to substances such as gasoline and ethylene decreases particle size through reducing soot precursor nucleation and growth [28].

Another finding of the current study was the impact of temperature on particle size. Qu et al. reported that an increase in temperature decreases surface tension and increases metallic particle size [29]. Although these findings were in good agreement with those of the current study, since the size of nanocarriers increased with temperature, the data were not convincing and may be attributed to reducing Gibbs free energy. In fact, increased temperature improves Gibbs free energy, while the system tends to decrease this level in order to improve thermodynamic stability; therefore, nucleation, growth, and aging processes are triggered.

In the current study, the increase in ethanol concentration from 3% to 5% and temperature from 22 °C to 60 °C respectively decreased and increased the particle size in drug nanocarriers through reducing surface tension and improving Gibbs free energy. Based on these findings, the increase in ethanol concentration from 3% to 5% decreased surface tension and consequently the particle size from 56.8 ± 0.1 to 47.9 ± 1 in a non-temperature setting. On the other hand, the increase in temperature (60 °C) decreased Gibbs free energy by improving nanocarrier nucleation and growth. At 5% ethanol concentration, the low surface tension overcame the increase in the Gibbs free energy of nanocarriers, and nanocarrier particle size was less than that of reported at 3% ethanol concentration.

Based on the current study findings, particle size influences ß1-integrin gene expression, and larger nanocarriers induce the overexpression of ß1-integrin in fibroblast cells. As mentioned earlier, ß1-integrin overexpression in fibroblasts leads to wound healing and increases the production of new ECM and type I collagen [12]. Moreover, soybean oil in nanoemulsions contains unsaturated fatty acids, which help change the spacing

of lipid bilayer in the stratum corneum and increase dermal delivery [5]. These findings may be valuable to pharmaceutical companies not to underscore the importance of nanocarrier particle size in the design of aging and wound healing products.

While larger nanocarriers (about 70–77 nm) induced ß1-integrin gene overexpression, smaller nanocarriers induced higher cell proliferation and cell viability, compared with larger nanocarriers. Brockbank et al. showed that ß1-integrin is not essential to proliferation of vulvar squamous cell carcinoma cells, while it is necessary for cell adhesion [30]. This finding was in agreement with the current study findings, which indicated that ß1-integrin overexpression was not associated with fibroblast cell proliferation. However, some studies reported the concomitant occurrence of ß1-integrin overexpression and proliferation of epidermal [11] and satellite cells [13].

Another important finding of the current study was the Bcl2/Bax ratio as a marker of anti-apoptosis. Although the results were consistent and larger nanocarriers induced less fibroblast cell apoptosis, they were also associated with ß1-integrin overexpression. It seems that 77-nm nanocarriers induced cell viability less efficiently than 50-nm nanocarriers in agreement with smaller nanocarriers in which they could induce higher cell metabolic assay or cell proliferation.

The increase of fibroblast apoptosis marker in smaller nanocarriers was due to the increased Bax ratio and the increased NFκB overexpression. Therefore, ß1-integrin overexpression leads to the downregulation of Bax as an apoptotic gene and NFκB as an inflammatory one. NFκB was involved in aging [14] and death of some cells; also, NFκB overexpression is higher in older skins than younger ones [15, 16], these findings were in good agreement with the current study data. However, others indicated that NFκB increment was in good agreement with cell proliferation of mammary epithelial cells and other cell types as observed in the current study.

Conclusion

In conclusion, curcumin nanocarriers of about 77 nm (without ethanol as a cosurfactant) induced more ß1-integrin overexpression, while addition of 3% ethanol decreased cell apoptosis, Bax, and NFκB genes. Based on the current study findings, a curcumin nanoformulation, containing 3% ethanol in the range of 77 nm, was more effective than a nanoformulation with a particle size of about 50 nm to decrease cell death (PI-positive and Bax/Bcl2 ratio) and expression of apoptotic (Bax) and proinflammatory (NFκB) genes; therefore, these formulations may be valuable in ageing and wound healing.

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Data availability Available upon the request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

References

1. Hoveizi E, Khodadadi S, Tavakol S, Karima O, Nasiri-Khalili MA. Small molecules differentiate definitive endoderm from human induced pluripotent stem cells on PCL scaffold. Appl Biochem Biotechnol. 2014;173(7):1727–36.
2. Hoveizi E, Ebrahimi-Barough S, Tavakol S, Sanamiri K. In vitro differentiation of human iPS cells into neural like cells on a biomimetic polyurea. Mol Neurobiol. 2017;54(1):601–7.
3. Weiss RA. Autologous cell therapy. Facial Plastic Surgery Clinics. 2013;21(2):299–304.
4. Thangapazham RL, Darling TN, Meyerle J. Alteration of skin properties with autologous dermal fibroblasts. Int J Mol Sci. 2014;15(5):8407–27.
5. Banerjee R. Overcoming the stratum corneum barrier: a nano approach. Drug delivery and translational research. 2013;3(3):205–8.
6. Sikora E, Bielak-Zmijewska A, Mosieniak G, Piwocka K. The promise of slow down ageing may come from curcumin. Curr Pharm Des. 2010;16(7):884–92.
7. Shen LR, Parnell LD, Ordovas JM, Lai CQ. Curcumin and aging. Biofactors. 2013;39(1):133–40.
8. Bala K, Tripathy B, Sharma D. Neuroprotective and anti-ageing effects of curcumin in aged rat brain regions. Biogerontology. 2006;7(2):81–9.
9. Thangapazham RL, Sharad S, Maheshwari RK. Skin regenerative potentials of curcumin. Biofactors. 2013;39(1):141–9.
10. Giangreco A, Goldie SJ, Failla V, Saintigny G, Watt FM. Human skin aging is associated with reduced expression of the stem cell markers beta 1 integrin and MCSP. J Invest Dermatol. 2010;130(2):604–8.
11. Raghavan S, Bauer C, Mundschau G, Li Q, Fuchs E. Conditional ablation of β1 integrin in skin. J Cell Biol. 2000;150(5):1149–60.
12. Liu S, Shi-Wen X, Blumbach K, Eastwood M, Denton CP, Eckes B, et al. Expression of integrin β1 by fibroblasts is required for tissue repair in vivo. J Cell Sci. 2010;123(21):3674–82.
13. Rozo M, Li L, Fan C-M. Targeting [beta] 1-integrin signaling enhances regeneration in aged and dystrophic muscle in mice. Nat Med. 2016;22(8):889–96.
14. Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, Chang HY. Motif module map reveals enforcement of aging by continual NF-κB activity. Genes Dev. 2007;21(24):3244–57.
15. Tilstra JS, Clauson CL, Niedernhofer LJ, Robbins PD. NF-κB in aging and disease. Aging and disease. 2011;2(6):449–65.
16. Kriete A, Kari C, Mayo KL, Yalamanchili N, Bender P, Rodeck U, et al. Cell autonomous expression of inflammatory genes in biologically aged fibroblasts associated with elevated NF-kappaB activity. Immun Ageing. 2008;5(1):5.
17. Saengkrit N, Saesoo S, Srinuanchai W, Phunpee S, Ruktanonchai UR. Influence of curcumin-loaded cationic liposome on anticancer activity for cervical cancer therapy. Colloids Surf B: Biointerfaces. 2014;114:349–56.
18. Hackley V, Clogston J. Measuring the size of nanoparticles in aqueous media using batch-mode dynamic light scattering. NIST Special Publication. 2007;1200:6.
19. Sun Z, Guo SS, Fässler R. Integrin-mediated mechanotransduction. J Cell Biol. 2016;215(4):445–56.

20. Himesh S, Sharan PS, Mishra K, Govind N, Singhai A. Qualitative and quantitative profile of curcumin from ethanolic extract of Curcuma longa. Int Res J Pharm. 2011;2(4):180–4.
21. Tavakol S, Saber R, Hoveizi E, Aligholi H, Ai J, Rezayat SM. Chimeric self-assembling nanofiber containing bone marrow homing peptide's motif induces motor neuron recovery in animal model of chronic spinal cord injury; an in vitro and in vivo investigation. Mol Neurobiol. 2016;53(5):3298–308.
22. Tavakol S, Jalili-Firoozinezhad S, Mashinchian O, Mahmoudi M. Bioinspired nanotechnologies for skin regeneration. Nanoscience in Dermatology. Elsevier. 2016:337–52.
23. Tavakol S, Nikpour MR, Hoveizi E, Tavakol B, Rezayat SM, Adabi M, et al. Investigating the effects of particle size and chemical structure on cytotoxicity and bacteriostatic potential of nano hydroxyapatite/chitosan/silica and nano hydroxyapatite/chitosan/silver; as antibacterial bone substitutes. J Nanopart Res. 2014;16(10):2622.
24. Tavakol S, Kashani IR, Azami M, Khoshzaban A, Tavakol B, Kharrazi S, et al. In vitro and in vivo investigations on bone regeneration potential of laminated hydroxyapatite/gelatin nanocomposite scaffold along with DBM. J Nanopart Res. 2012;14(12):1265.
25. Tavakol S, Mousavi SMM, Tavakol B, Hoveizi E, Ai J, Sorkhabadi SMR. Mechano-transduction signals derived from self-assembling peptide nanofibers containing long motif of laminin influence neurogenesis in in-vitro and in-vivo. Mol Neurobiol. 2017;54(4):2483–96.
26. Tavakol S, Saber R, Hoveizi E, Tavakol B, Aligholi H, Ai J, et al. Self-assembling peptide nanofiber containing long motif of laminin induces neural differentiation, tubulin polymerization, and neurogenesis: in vitro, ex vivo, and in vivo studies. Mol Neurobiol. 2016;53(8):5288–99.
27. Chin SF, Azman A, Pang SC. Size controlled synthesis of starch nanoparticles by a microemulsion method. J Nanomater. 2014;2014:9.
28. Salamanca M, Sirignano M, Commodo M, Minutolo P, D'Anna, A. The ethanol effect on particle size distributions in ethylene premixed flame.
29. Qu Y, Yang H, Yang N, Fan Y, Zhu H, Zou G. The effect of reaction temperature on the particle size, structure and magnetic properties of coprecipitated CoFe 2 O 4 nanoparticles. Mater Lett. 2006;60(29):3548–52.
30. Brockbank E, Bridges J, Marshall C, Sahai E. Integrin β1 is required for the invasive behaviour but not proliferation of squamous cell carcinoma cells in vivo. Br J Cancer. 2005;92(1):102–12.

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