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RESEARCH ARTICLE

Synergetic effects of Chick Embryo Extract and Gold Nanoparticles for Matching Skin Flap Regeneration

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ABSTRACT

Background and objective: In the present paper, impacts of gold Nanoparticles (AUNPs) and Chick Embryo Extract Therapy (CEET) were investigated on the wound healing promoting properties that match the dynamic healing process of skin flap regeneration.
Methods: AUNPs were developed using the high-intensity short-pulse beams mode of neodymium-doped: Yttrium, Aluminum, and Garnet photoablation method. Also, 45-µg extract from a 4-6 day chick embryo was used as a protein source for CEET. Dorsal Random Skin Flaps (RSFs) were created in forty-five Albino Wistar rats and they were accidentally assigned to 3 groups; G(C); group did not receive any treatment (control), G (AUNP); treated only with AUNPs, and G(CE+AUNP) the rats treated with AUNPs and CEET. During a random skin flap animal model, AuNPs and CE were used as solutions and convenient to deliver into the skin flap. The skin flap survival rate was evaluated in the following by a camcorder, and tissue studies were done at days 3, 6, and 9 after surgery by the hematoxylin & eosin and Masson trichrome staining.
Result: Rats treated with the AUNPs and CEET exhibited remarkably increased flap survival than the others. Tissue studies showed that AUNPs and CEET caused induction of forming the new blood vessels and stimulated the inflammation-causing reaction in an initial phase of wound healing.
Conclusion: Using AUNPs with CEET increases the potential for a dynamic recovery of skin flap regeneration.

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INTRODUCTION

Injured tissue repair could also be an active and complicated procedure. In the primary phase of skin flap healing, tissue endures impaired cell stability and death due to tenderness, inadequate blood and oxygen supply thus, within reproduction phase; the cells are reproduced fast when the endothelium-related cells show sufficient blood development, whilst the collagen-producing cells and mesenchyme-related stem cells contribute to rejuvenation and renewal[1]. At last, in reworking phase, the tissue experiences the rebuilding and development in its form and arteries-related system, as a result of which, it consequently retrieves its regular performance within months or years. Several wound treatments are proposed, that is, pharmacotherapy[3], cellular therapy[4], extracorporeal shock wave therapy[5], and negative pressure wound therapy (NPWT)[6], exogenous factors stimulation therapy[7] also as protein therapy[8]. Growth factors transport the signals among the cells for induction of reproduction or directed migration of the cells towards a specific environment, consequently leading to the ECM development and formation of new blood vessels. Identifying the fibroblast growth factor (FGF) in tissue extracts of embryonic organs, and thus the correlation of its presence with particular developmental events is implicational a task for this protein in organogenesis[9]. Vital

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fibroblast protein appears broad multiplication in created tissues, which has been affirmed by biochemical, and immunological examination of tissue extricates. The basic fibroblast growth factor (bFGF) is provided by the tissues of living organisms similar to the chorioallantoic of the chick embryo as exogenous, suitable for enhancing angiogenesis. A big number of positive comes about of Chick fetus extricate treatment on repairing are detailed in vivo or in vitro considers [10]. Chick embryo extract therapy (CEET) can increment cellular replacement, diminish cell death, induce the formation of new blood vessels, reestablish the blood circulation in the smallest blood vessels, also improve the injury healing [11]. A great amount of reactive oxygen species causes extension of oxidation, impairing the rejuvenation procedure. Thus, antioxidant operators have been assumed to decrease the oxidation level and debilitate harming impacts by the reactive oxygen species that damage the repair. ROS is created as a dynamic by-product of cellular digestion system, which capacities as cellular signaling pathways[13]. However, an abundant combination of reactive oxygen species and organic molecules hurts the deoxyribonucleic acid, ribonucleic acid, protein, cell function, also restrains the development[14]. As an example, within the first phase of accidental skin flap regeneration, ischemia leads to inadequate oxygen supply for the flap, restoration of blood flow after the blockage, and tenderness, specifically within the points away from the torso. The adverse condition of inadequate oxygen supply prevents the flap to be reconstructed fast in primary phases. One of the types of antioxidants called nano-materials is a metal material that uses metrics, basic properties, and its implementation as an antioxidant. This nanomaterial has recently attracted the attention of researchers. It is believed that nanomedicine in cases such as drug delivery, regenerative medicine, and imaging is widely used[15]. Gold nanoparticles (AuNPs) are an eminent source of intrigued since of the new features like dimension-associated electric[16], optical[17], synthetic attractive features, which are possibly valuable for natural purposes[18].AuNPs have strong antioxidative impacts in extinguishing free radicals like DPPH, Gracious hydroxyl, H2O2, and NO [19]. Oxidation-inhibiting action in gold NPs concerning free radicals altogether depends on the exact surface [20]. Sphere-shaped gold nanoparticles have an outsized zone, which wraps up amid a high inclination to effectively acknowledge the negatively charged subatomic particle and reactive oxygen species for their rummage or shutting down. Consequently, AuNPs are considered as powerful oxidation-inhibiting agents and have a significant role in wound healing[21]. However what isn't acknowledged, the prospective of nanoparticles related to types of growth factors something like that embryo organs. So, this study was conducted to evaluate the results of the active recovery method of skin flap repair in the rat model exposed to therapeutic AuNPs and CEET by 45µg.

MATERIAL AND METHODS

Animals

45 Albino Wistar rat with a weight of 250-250 gram were operated in the present research. During a room where light (12 hours light cycle), temperature, and humidity were controlled, the rats were at the same time placed in separate plastic cages and special diet and water were provided for them as much as desired. This study was approved by the LUMS with the Ethics Code of IR.LUMS. REC.1397.096.

Synthesis of Gold Nanoparticles (AuNPs)

This procedure was performed supported laser ablation on the gold disc (with a purity of 99.99%). Before laser irradiation, the golden disc was immersed in the Branson high- frequency pressure (sound) waves- cleaner for twenty min and therefore, the golden disc was cleaned after 20 minutes.

To prevent organic matter interactions, a golden disc was rinsed using the propanone. For the assembly of spherical gold nanoparticles, C12H25SO4Na was prepared by laser ablation of Au disc by laser in a solution of dodecyl sulfate. Within the above work, the Quanta-Ray GCR-170 Nd: YAG laser (wavelength 657 nm; pulse time interval: 10 µs duration and 10 Hz repetition speed) was used. After 22 min of photoablation, the solution color was turned into vermilion, indicating the presence of AuNPs[22] .

Evaluation Methods (DLS and Zeta potential)

Dynamic Light Scattering (DLS) was used to analyze the size of the AuNPs distribution. The DLS method used a Brownian motion nanoparticle (NP) in a solvent to measure their size distribution. Laser throwing into the particles and observing

the scattering light in a specific direction made it possible to analyze the NP size distribution. Also Zeta potential is related to various theories (Smoluchsky or Huckel) that are directly associated with the mobility of NPs. Using the Doppler Electrophoresis Laser technique, the NPs were measured to determine their motility (Genesis of Cordouan Technologies Company, French).

Providing the EE and Bradford Protein Assay

Fecundated eggs (mean mass of sixty grams) were provided by a trading chicken-breeding place (Toyoorbarekat, IRAN) and were kept in a simulating-device of avian incubation under normal situation (hotness and water vapor concentration of 37.5 degrees of Celsius and 65%, correspondingly, adjusted every hour) till they were used. After4, 5, or 6 days of brooding, eggs were accidentally chosen and unclosed to obtain their contents. Two-hundred microliter of every specimen was made uniformed in the PBS solution by a homogenization device (Qiagen GmbH, Hilden, Germany) and then, it was centrifuged at a speed of two-thousand revolutions per minute for ten min. The liquids overlaying the sediment were gathered and became weaker ten times by adding the weaker-making solution to analyze the protein. Chicken fetuses were selected to be used later if their biology-related dynamic constituents were at the maximum amount. The suspensions containing cell constituents in the chicken embryos were lyophilized at −80 degrees Celsius and were kept at −70 degrees of Celsius to extract the protein and perform other animal tests. The amount of protein in chicken embryo extract was measured following the Bradford protein assay. CEE protein was defined from absorbance reading at 595 nm, with Bovine Serum Albumin (BSA) as a standard [23].

Creating random skin flaps in animals

Each of the rat was anesthetized through the infusion of ten mg/kg of KTX (2.5 ml of Ketamine, 12.5 ml of ketamine, Xylazine analog). The back hair was shaved employing a sterile blade. A 1.5 ×5 cm (1: 3) random skin flap was created within the back of the rat. Then, 45 rats were randomly assigned to 3 treatment classes: the rat received no treatment as G (C) placebo A (Aunp) endured topical cure with AuNPs. Group G (Aunp + CE) was treated with AuNPs, including CEE. Zero operation day was considered. In-group G (Aunp), 0.1 ml of AuNPs sol was applied to the flap employing a pipette, therefore the rat was placed in separate cages. In group G (Aunp + CE), 0.1 ml of AuNPs Sol was poured on the flap and given 10 minutes to penetrate the AuNPs nanoparticles into the flap bed. 45 µg of CEE was infused in the outer covering of the body before the replacement of the flap in the primary location. Treatments were performed only on day zero and, therefore, the animals were clinically examined during 9 days post-operation[24].

Assessment of flap survival area and Sampling

Results of the examination were assessed every day using the Canon EOS 1200 D camcorder post-healing. The area for the flap survival was calculated with a photo analyzer (Image 1/46r, NIH, USA). 5 rat in every treatment class were slaughtered and their bodys' outer covering tissues were gathered for assessment of tissue studies in days 3, 6, and 9. Outer covering tissues were placed in 10% NBF for three days at room temperature. Then, they were shaved, rinsed, and their water was removed by increasing amounts of ethyl alcohol (fifty, seventy, ninety, and one hundred %), and were cleansed by xylol and placed in petroleum wax. 3-mm sections were prepared from the outer covering using the microtome and colored by HE stain and TRI. Slides were seen by the Light Microscope (LM) (Leica Germany) using a camcorder (MU035)[25].

Statistical analysis

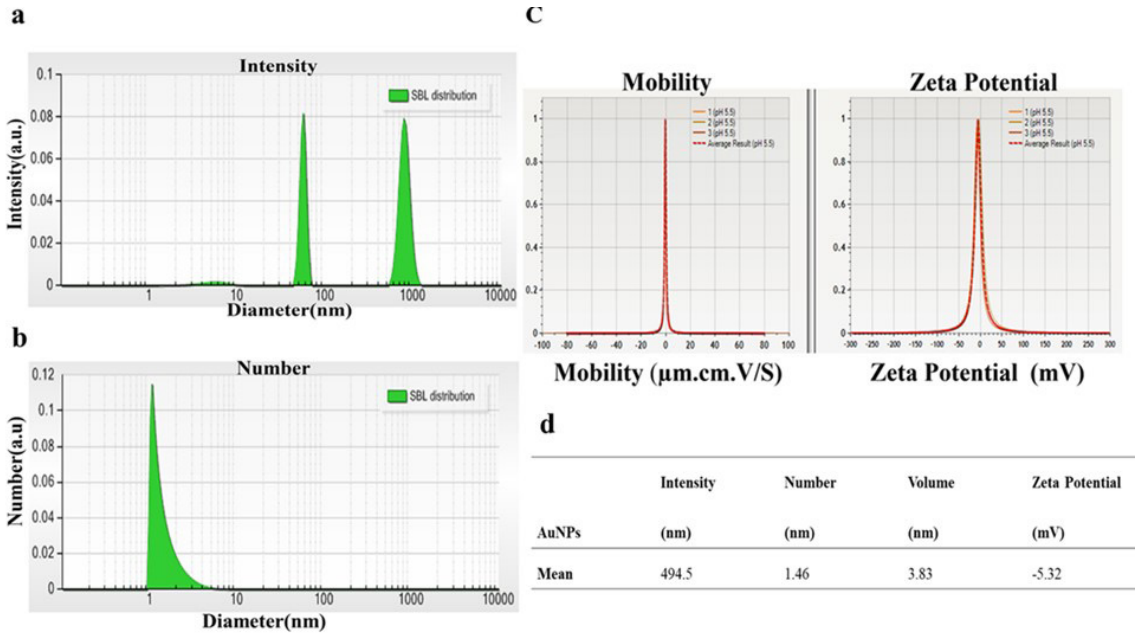
Results are presented as mean values ± standard error using Graph pad prism version 8.0.Statistical comparisons between two groups with t-tests or among greater than two groups with a one-way ANOVA. Results compare were considered significant when $P < 0.05$.

RESULTS

The AuNPs (Fig. 1a) shows the gold in terms of intensity and number of particles. The DLS test sample was presented with the SBL analysis.

Diagram analysis (Fig. 1(b)) showed the mean particle dimension for gold NPs with the thinnest dimension dispersal.

The total protein concentration in the CEE was 32.21 µg/ml. it had been isolated from4, 5, 6-day-old embryos. Consistency within the survival area rate increased significantly within the Aunp+CE group in comparison to the control group. The utilization of mixing AuNPs colloids in conjunction



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	Intensity	Number	Volume	Zeta Potential
AuNPs	(nm)	(nm)	(nm)	(mV)
Mean	494.5	1.46	3.83	-5.32

Fig. 1. (a)Intensity and (b) Mobility; DLS diagram of nanoparticle gold hydrodynamic diameter after surface modification gold nanoparticles (AuNPs), (c) Zeta potential diagram of colloidal gold nanoparticle analysis (d) Mean of DLS and zeta potential result of gold nanoparticles with each surface modification.

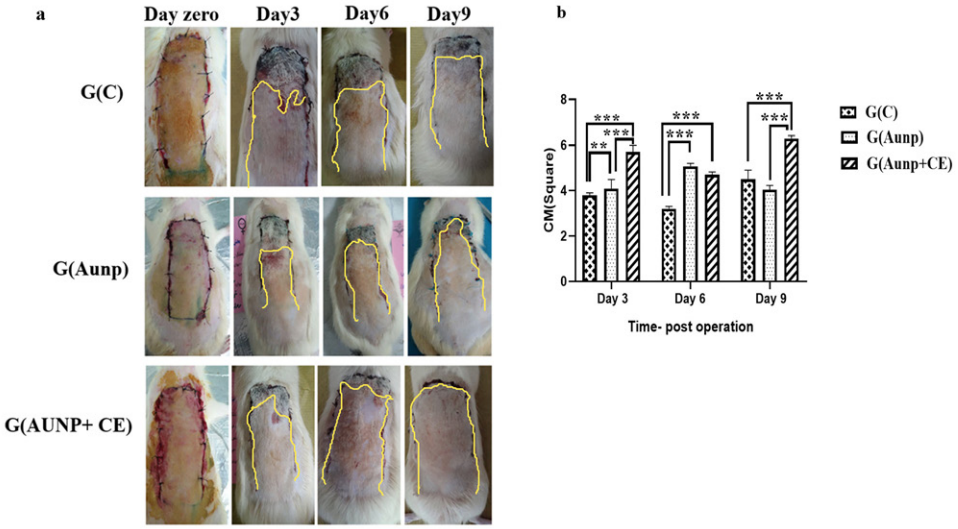

Fig. 2. Morphology of skin flaps survival a different time point. The whole survival area of flap is outlined in a yellow line in experimental groups: control, Aunp, and Aunp +CE groups. The Aunp +CE treated group had a far better survival rate of skin flap than the opposite groups a. Morphology of skin flaps over 9 days post-operation. b. Quantification of percent survival skin flap. (*P < 0.05).

with CEET for the dynamic healing process of skin flap regeneration. The result of the survival rate of the flap of the groups tested is shown in Fig. 2. The survival rate of the flap and the survival of the tissue in groups G (Au) and G (Aunp + CE) 2 were higher compared to the group without intervention. The highest survival area was found in treatment class G (Aunp + CE), which was about 1.25 times higher than the control class within the study period. Optical microscopic observations on skin biopsies were performed after staining of hematoxylin-eosin and trichrome on days 3, 6, and 9 (Figs. 3

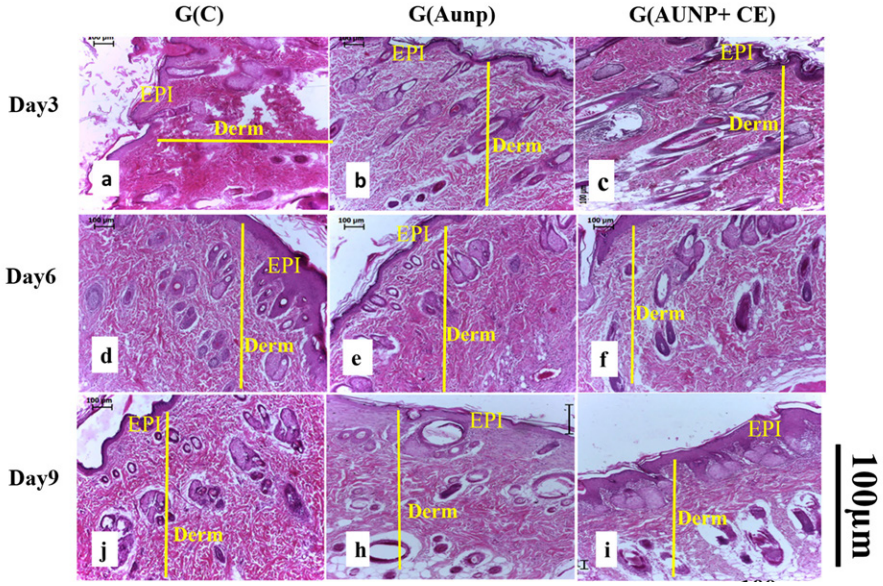

Fig. 3. Image of skin flap at various time points marked with epidermis (EPI), dermis (DERM) (a, d, j) control class; (b, e, h) those treated with gold NPs and no CEET; (c, f, i) by CEET are appeared by HE stain on the third, sixth, and ninth days at an enlargement of 100× (Scale bar 100 µm)).

and 4). On the third day, skin substrate, the narrow substrate of the epidermis, are steadily formed in all treatment classes, (Fig. 3 (a), (b), and (c)). In G (Au) and G (AuNPs + CE) treatment classes, progressed blood vessels were further repaired.

There was a large number of fibroblasts in the presence of blood vessels in the group (Aunp + CE) and is collected. These newly formed blood vessels are connected by an organized microscopic network, as shown in Fig. 3 (b), (c).

The penetration of inflammatory cells in a G (Aunp + CE) group was noteworthy on the third day, although it was mild for the group (gold NPs) and control. Within Masonic trichrome slides, an agreeable collagen statement was observed within the control group within the center portion of the wound (Fig. 4 (a)), while thin collagen within the flap was observed for group G (Au) (Fig. 4 (b). While in group G (Aunp + CE), thick collagen is detected, which is revealed inside the center of the wound as revealed (Fig. 4 (c)). On day 6, AuNPs revealed less influence than AuNPs with CEET, which is revealed in slower wound healing and moderate penetration in granulation tissue (Fig. 3 (b)), whereas wound epithelialization is completed using the gold NPs and CEET, (Fig. 3 (c)). A thicker bond layer is additionally shown and an epidermis is made. Additionally, severe inflammatory reaction was seen for controls and those treated with G (gold NPs) (Fig. 3 (d), (e)), but in G (Aunp + CE) the group reduced slightly on the sixth day. For G (Aunp + CE), the bonding substrate was unevenly formed, so the follicles, fur small secretory cavity, and fatty group of cells are formed. Additionally, dense collagen was seen from mainstay the lowest to the highest of the injury for class G (Aunp + CE) (Fig. 4 (F)). On day 9, all groups revealed fine within the flap region, and tissue association was formed again irregularly. But, wounds treated with AuNPs, including CEET, are well-classified skin and the sweat performance was achieved, follicle was formed, thus an outsized volume of collagen that's located within the flap compared to other groups. Microscopic observations revealed that the group treated with AuNPs, including CEET, had an optimal effect on the method of dynamic skin flap rehabilitation than those which just received gold NPs.

5. DISCUSSION

One of the mediators included in cellular signaling that protects against forceful pathogens is low levels of ROS [26]. In any case, over the top generation of ROS eventually impedes the healing process of wound Recovery by repressing development, causes cellular harm, which closes

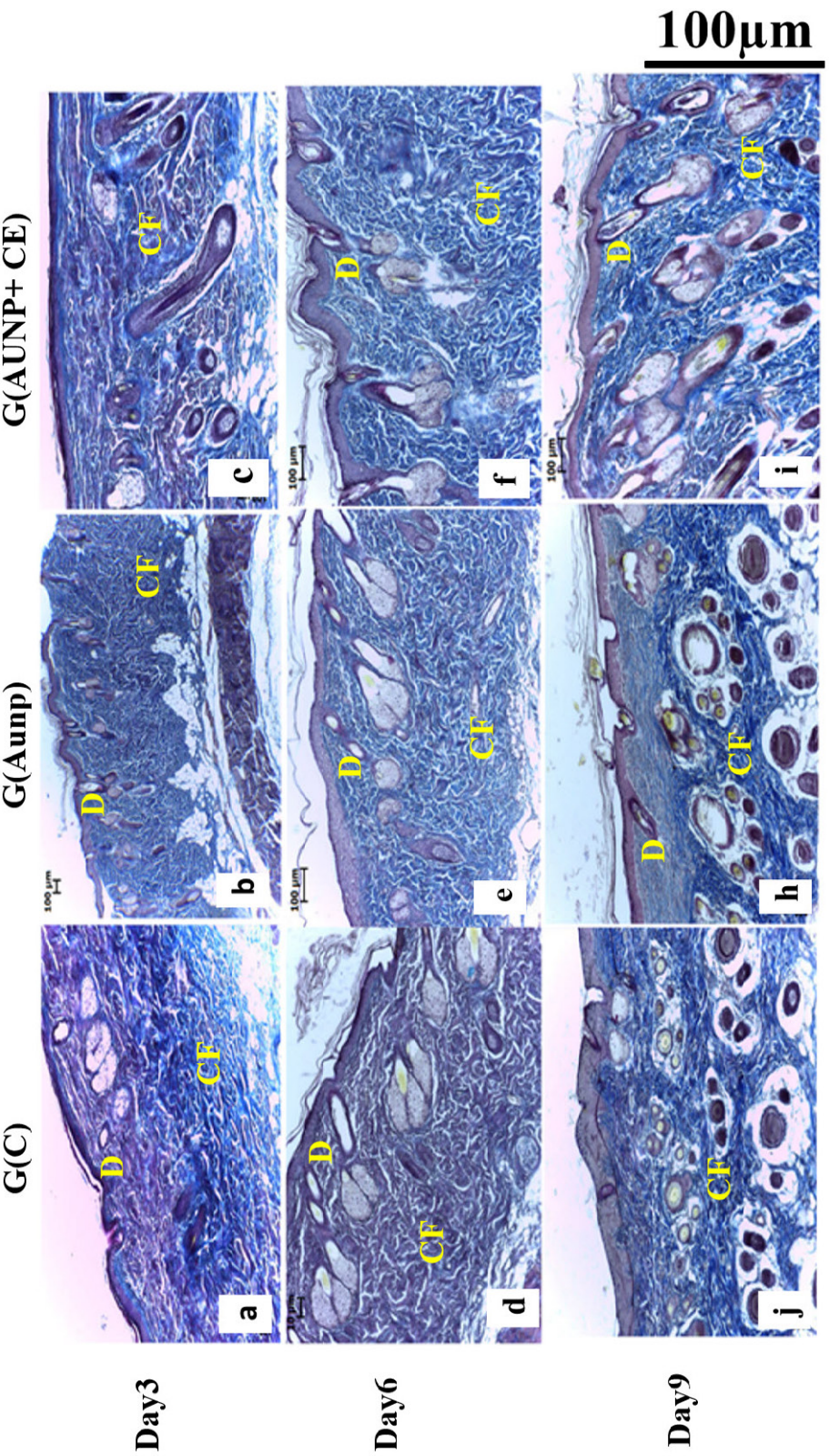

Fig. 4. Image of skin flap at various time points marked with collagen fiber (CF), duct of sweat gland (D) by trichrome mason in stained on the third, sixth, and ninth days (100x magnification and scale bar100µm).

up in destitute neovascularization[27] .Wound healing is regularly advanced through the nearness of antioxidants through the diminishing degree of reactive oxygen species. Au NPs possess fabulous oxidation-inhibiting features including biocompatibility, great surface reactivity, along with being non-toxic. In this way, it's been conveyed to the intrigued of conceivable outcomes for restorative purposes. BarathManiKanth et al., used the AuNPs to dissolve toxicity of gold NPs to human body parts inside the liver, renal, and Pulmo. No significant form-related variations were observed in the body organs subjected to the treatment and the digestion system of proteins was normalized for rescaling the collection of reactive oxygen species and improving the cell function. Researchers identified that gold NPs possess anti-inflammatory, antioxidant, antimicrobial, and anti-angiogenesis features for strengthening the expansion of collagen-producing cells and reducing the cell death during the injury improving[28].Some study Examined the results of gold NPs and other oxidation-inhibiting operators in local uses for injuries caused by diabetes[29]. The local usage of gold NPs essentially quickened rehabilitation forms via a response to inflammation and activated the unused veins to develop on-secretion veins. Another comparable work utilized gold NPs covered for treating the skin injuries in a rodent model[30]. They showed that the speed levels of injury healing are four folds higher in the treated class compared to the others. Moreover, those subjected to gold NPs treatment had an enormous increment in an expression of collagen, VEGF, Ang-1, and Ang-2, suggesting that gold NPs therapy quickened the formation of new blood vessels during injury recuperating, which are consistent with our study showing that the two gold NPs and CE therapies quickened the survival zone and positively influenced the formation of new blood vessels on the third day. Protein extracts were activated due to functional constituents in chicken embryo supporting healing. Both acidic and basic fibroblast growth factors (aFGF) and bFGF are identified in extracts of embryonic tissues, suggesting that these growth factors could also be involved within the regulation of developmental processes. The presence of FGF within the embryonic brain (aFGF- like 24 ng/g; aFGF and bFGF: 18 ng/g) and kidney (aFGF: 25 ng/g) has been correlated with vascularization of those tissues. This is often according to the well-known angiogenic activity of FGF in vivo model systems[31]. The communication of AUNPs and tissues might remain distressed from numerous elements like platelet-derived protein (PDGF), *basic fibroblast growth factor*, and (EGF) in a local application for foot sores caused by diabetes [32].

Growth factors do not only accomplish the injury rehabilitation. They also majorly contribute to tissue conservation and therefore continuance of intracellular signal transduction [33]. In past think about, CEET was found to be ideal for incitement impacts with stem cells at a dosage of 6×109 bone marrow mesenchymal stem cells (BMMSCs) were suspended in 1.0mL DMEM. Subsequently, identical CEE features were utilized to induce the injury while applying the AuNPs. CEET possesses critical inducing impacts on decreasing the sever, reaction[34], cellular movement, and multiplication of keratin-producing epidermal cells. Also, CEET can reduce the assembly of reactive oxygen species for rescaling oxidative stress by decreasing the action of inflammatory cells or inducing the oxidation-inhibiting enzyme[23]. Researchers reported that Growth factors influence the cell function even at minuscule amounts; often done through sending specific biochemical signals to special triggered cells through special wall receptors[35]. Growth factors stimulate several activities through binding to special receptors on the cell membranes in triggered cells [36].

Anti-inflammation impacts of CEET are talked about in various ponders and do not suppress the event of irritation, but or maybe it had been initiated early and abbreviate the inflammatory stage [37]. In our histological investigation, the gather of G (Aunp+CE) highlights a direct severe reaction on the third day, along with diminishing to mellow at the sixth day. Conversely, the controls and those treated with gold NPs have mellow incendiary activity on day 3, but it gets to be extreme on day 6. The prove recommends that CEET can trigger the fiery stage early[38]. The treatment utilizing AuNPs bolstered high-intensity short-pulse beams *mode of neodymium*-doped: Yttrium, Aluminum, and Garnet photoablation incredibly quickened rehabilitation procedure, a diminished sum of microscopic organisms, along with the manifestation of hair-follicle recovery. Herein, there was an exception such that, utilization of round gold NPs and CEET was prepared to advance the recuperating process of skin flap recovery through the shortening of the

fiery stage, advanced angiogenesis, and collagen generation. Be that as it may, there's a shortage of studies on the employments of AuNPs and CEET in reconstruction therapy, thus there is a need for adjuvant therapies for opening idle reconstruction pathways involved in oxidation-inhibiting and stimulating reaction.

6. CONCLUSION

Herein, the impacts of round gold NPs along with CEET were investigated on improving skin flap. The application of AuNPs with and without CEET on the energetic healing process of skin flap recovery, skin injury showed that the two treatment approaches possess useful impacts on injury rehabilitation than the non-treated class. Subsequently, it is recommended to apply both gold NPs and CEET for successfully curing injury and obtaining better outcomes in tissue studies. Advanced directed studies inside and outside the body on NPs and CEET combinations are required for increasing the information regarding precise instruments in use.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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