

Synthesis and characterization of Lipoic Acid–Peptide Conjugates and Their Effect on Collagen and Melanogenesis

Assistant Lecturer Khalidah Khalaf Jabbar

Medium Ghazali Boys School, Directorate of Education, Rasafa north of Baghdad, Iraq

Abstract

Current study aims to discovered new example of Lipoic Acid Epiphytes (LA), and they had anticonvulsive and anti-aging properties added. These multifunctional molecules had been organized via binding fats lipid (LA) with Pentagram KTTKSMore important than the pigment breakdown by the peptide is the breakdown of melanin, which in this case inhibits synthesis or Pent peptide on my own. Importantly, the conjugate show does no longer have cell toxicity at excessive attention. LAeKTKS and LA ePEGekKTKS additionally save you expression of the metalloproteinase-1 matrix of ultraviolet radiation through as much as forty-nine .five% and sixty-nine .five% at zero. Five mm, respectively. Peptide comparisons stimulate the biosynthesis of collagen in fibroblasts extra efficaciously than the mom molecules. These statistics imply that peptides might also include cosmetics observe as anti-melanin and anti-getting older retailers.

Keywords: PEGylatedlipoic acidester, Peptide Collagen, Antimelanogenic effects.

1. Introduction

Most of the ECM (extracellular matrix) tissue is made up of individual microscopic openings, including pores and skin, yet it serves as the systemic backbone because of many cosmetic and dermatology applications.. ECM proteins, as consist of collagen and-collagen fibers, lead a central characteristic among telephone migration, proliferation, yet gene system within the direction regarding shock restoration. Collagen-is the hugest among human beings [2]. The collagen fibers provide tensile government yet give as allow individuals together with pores and pores and skin in accordance with structure set up systems. Reduce ECM additives together with collagen breach and harm [3].

The immoderate accumulation of glycosaminoglycan's (GAGs) results in wrinkles that accompany growing older. These traits have led researchers to cognizance at the significance in growing old. The procedure has much chemical merchandise and compounds especially to stimulate ECM bio-synthesis to enhance the growing old pores and skin situation. In unique, a few oligopeptides carry out critical capabilities within the human frame and inside the pores and skin as a biologically lively message which include hormones, neurotransmitters or neurotransmitters[4]. Some beauty peptides had been evolved to enhance the paintings of pores and skin body structure with the aid of appearing as anti-getting older sellers or moisturizing remedies. And beauty medicinal drug. For instance, the hex peptide of Ala-Gly-Val-Gly-Val-Pro (AGVGVP) stimulates the manufacturing of fibroblasts of humpores and skin at the same time as

concurrently regulating elastin expression. The collection of peptides of Tyr-Arg Asp-Ala (YYRADDA) inhibits precollege-C proteins [5].

Which clears the seasoned-kind precollege, main to a lower in the manufacturing of latest collagen by way of fibroblasts. The pores and skin offer a bodily and chemical barrier in opposition to the damaging consequences of the outside surroundings; normal peptides have an exceedingly low fee of bioavailability because of enzymatic digestion and different inner elements, inclusive of half of around lifetime measured in hours or mines[6]. Therefore, many compounds and penetration enhancers were synthetic to boom protection, balance and transport of peptides throughout the pores and skin. These consist of substitution of amino acids D-L amino acids to less publicity to positive and include into enhance 1/2-existence, covalent amendment through polymer coupling lessen enzymatic digestion, kidney elimination, and immunity. The peptide is the maximum extensively promoted within the discipline of topical cosmetics[7].

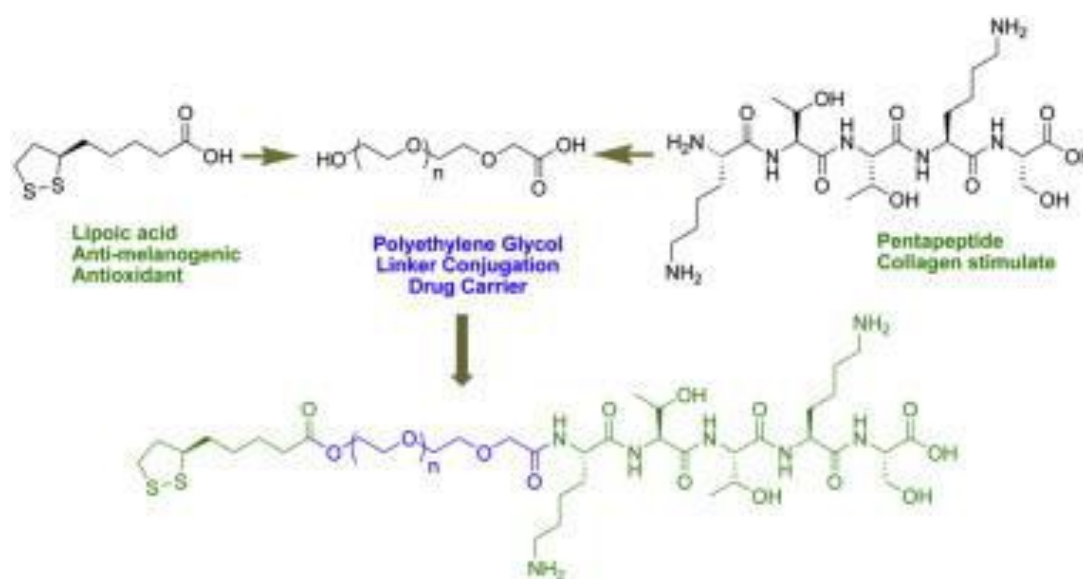


Figure 1. to look in regard to LApeptide conjugates, and with regard to follow up with the Cleavage after preparation.

The utility is KTTKS which includes 5 amino acid residues (for this reason pentapeptide) that own the capability to decorate remodeling by using mobile procedures, together with growing ECM manufacturing. However, there are numerous issues to take into account whilst KTTKS as nearby additives, particularly their passive penetration and penetration via the pores and skin [7]. The techniques to be had to enhance neighborhood shipping is the advanced use of chemical, including fatty acids, carried out to KTTKS as a manner to enhance its transport throughout the pores and skin, together with attaching a lipid-loving organization by means of forming an N-palmitoyl-KTTKS, A fundamental issue in Matrixes. Recently, a brand new compound based totally on KTTKS and ascorbic acid, referred to as the strong ascorbic pentagon, has been advanced. It is higher to stimulate human fibroblasts in vitro to supply greater collagen than mom compounds on my own[8]. Chemical change with polyethene glycol (PEG) can enhance drug overall performance via chemical and organic balance, in addition to drug penetration inside the manipulated pores and skin.

Biological consequences. Its antioxidant residences aren't constrained to even blockage of dopamine, however additionally expand to regulate the expression of melanin enzyme. And consequently, it's far an ability candidate for the remedy of pores and skin most cancers helper. The layout and synthesis of hybrid molecules containing pharmaceutical pills at the identical scaffold is a nicely-mounted technique to synthesizing greater powerful pills with double interest. Using this method, LA has been extensively used to synthesize many unions and has a multifunctional hobby[9]. In this have a look at, we recommended a synthesizing novel LA peptide. Comparisons (1e2) wherein pent borate is connected to lipid (LA). We defined synthetic development in LAe (1e2) peptide complexes and in comparison their organic hobby with the unique hobby of reporting. We have proven that peptide bindings drastically inhibit the synthesis of melanin higher than the authentic molecule. In addition, organic interest in fibroblasts (HDFs), together with the inhibitory impact at the expression of the metalloproteinase-1 matrix and the catalytic impact of collagen synthesis. Our findings recommend that peptide bindings can be a strong candidate for anti-growing older ant aging sellers[10].

1.1 Blood collection

Blood samples had been acquired from the cuboid vein. Anticoagulants (EDTA K2) within the morning inside the NT institution. In the instructor's guys (TCON and TALA), blood samples were gathered earlier than. Exercise, at once after the final touch of workout and after 24 hours of the healing duration[11]. Samples are located straight away at four ° C after series. Within 10 mines, the blood samples have been centrifuged (2500 g, 10 min, four °C). Plasma pills have been saved at -20 °C. The erythrocyte element became suspended 3 instances in bloodless isotonic saline and centrifugal (2500 g, 10 min, four °C). Red blood cells had been saved washed at -20 ° C till evaluation. All samples had been analyzed inside 7 days.

1.2 Isometric/ Isokinetic Practice

The exercise protocol consists of voluntary isometric constriction for 10 seconds (most overall performance). Thigh muscle businesses at 30 levels and seventy-5 stages of lap bend on Biodex 3-Dynamometer System [12]. The subjects completed the isometric series with torque pinnacle. The right limb on the quantity of 195.1 ± 28.6 Nm (ext. M) and 154.9 ± 26.3 Nm (bending) at knee bend 30 °, 357.4 ± 46.2 Nm (extender) and 127.6 ± 28.4 Nm (m) At knee bend seventy five °, left limb at 198.7 ± 33.8 Nm (ext. M), 138.9 ± 27.1 Nm (flex) at 30 ° knee bend, 335.1 ± 55 Nm (extender) and 123.7 ± 24 Nm (bend) while bending the knee seventy five stages. The isokinetic series turned into then carried out at 60, one hundred twenty, one hundred eighty, 210 and 450 ranges angular velocities. Figure 1. The isokinetic series changed into positioned in a sitting function with the knee at ninety stages of bending and repeated till fatigue. The height torque turned into accumulated, the most torque reaches time, general paintings, common energy, and common torque most torque of Biodex all through muscle overall performance measurements. Topics had been recognized with every of the workout protocols earlier than facts series commenced[13].

2. Results and Discussions

2.1. Chemistry

The well-known approach for the synthesis of leptide bindings (1e2). There are 3 components of the synthesis within the 1 (LAePEGEKTKS) assemblies. The element is the synthesis of the by-product LA PEGylated LA ester derived four (LAePEG). The 2nd element is the synthesis of peptides in stable section chemistry. The 0.33 component is coupling LAePEG and pent peptide. In the case of LAeKTKS (2) mixtures, the primary component is peptide synthesis; the second one is the affiliation of LA and pent peptide. The LAePEG synthesis changed into finished the usage of a pre-evolved method (Figure 1). A hydroxyl a hydroxyl-u-carboxyl PEG (HOePEGeCOOH) changed into received thru an easy system beginning from commercially to be had homobifunctional PEG, carries one step chromatography and chemical change. The heterobifunctional PEG has an unmarried peripheral hydroxyl institution to be had for LA, hence suppressing the formation of byproducts and offering a purposeful organization of carboxylic for coupling with pentagon peptide[14].

Vehicle characterization changed into done the usage of the NMR approach. NMR facts from LAePEG (four) suggest that once LA is connected to a PEG, the ensuing spectrum is a simple aspect of the beginning cloth. The major contribution of the PEG institution seems like a massive extensive range in three.5e three.7 ppm with an extra trigon proton at four.2 ppm and the LA bar is proven at 1.4e3.2 ppm. Since the LAePEG spinoff has been nicely laundered numerous instances, the end result of nuclear magnetic resonance (NMR) suggests that LA efficiently connected to PEG. Through the response of Astra. The magnetic resonance imaging (HMR) spectrum of 1H for the same assessment 1 became just like that of the accompaniment 2 besides for the big multiplex at three.5e3.7 ppm constructed into the PEG backbone as formerly mentioned in LAePEG (four). These exclusive echoes lead us to discover unequivocally the compounds organized on this research[15].

2.2. Biological Assessment

The water solubility of leptide aggregates (1e2) became greater than a hundred mg / mL. Cellular toxicity changed into detected from LA peptide comparisons by means of assessing the consequences of mobile survival.

The maximum non-poisonous awareness of survival of B16F10 pores and skin most cancers cells, which changed into the best dose examined and did now not lessen the validity of one hundred% of B16F10 pores and skin most cancers cells after forty eight hours at 37 °C for incubation, (see Table 1). The indicated values. The most non-poisonous awareness of mobile survival became that leptide unions (1e2) have been much less poisonous to B16F10 cancer cells than LA cells. LA confirmed a huge cytotoxic impact on cellular survival, down To zero.1 mm, even as enormous variant became discovered within the LAe (1e2) peptide complexes. The impact of dose-based LA peptide coupling at the wide variety of human pores and skin fibroblasts (HDFs) turned into investigate.

The cells had been incubated with zero. Five, and five mm samples for forty-

eight hours. As proven in Figure 1, LApeptide unions inspired fibroblast proliferation interest, and the quantity of cells elevated by using most of 24.5% for LAeKTKS and thirteen. Three% for LAePEGekKTKS at zero[16]. Five mm, respectively. The most proliferation results have been 12.9% for LAePEG and thirteen. Four% for KTKS at five mM, respectively. LA did now not have an effect on cellular proliferation at low attention (zero.1 mM) and to be poisonous to cells in tradition at excessive concentrations [17].

Table 1. The high nontoxic concentrations of LA, LAePEGKTKS and LAeptide conjugates (1e2).

Compound	The maximum amount of nontoxic concentrations (mM)
LA	0.1
LAePEG	5
LAePEGekKTKS	2
LAeKTKS	1
KTKS	5

The water solubility of leptide aggregates (1e2) changed into extra than one hundred mg/ mL. Cellular toxicity became detected from LAeptide comparisons by way of assessing the results on mobile survival. The maximum non-poisonous awareness of survival of B16F10 pores and skin most cancers cells, which changed into the best dose examined and did now not lessen the validity of one hundred% of B16F10 pores and skin most cancers cells after forty-eight hours of incubation at 37 ° C, (see table 1). The indicated values. The most non-poisonous awareness of cellular survival became that leptide unions (1e2) have been much less poisonous to B16F10 cancer cells than LA cells. LA confirmed a full-size cytotoxic impact on cellular survival, down to zero.1 mM, even as extensive variant changed into located inside the LAe (1e2) peptide complexes. The impact of dose-established LAeptide coupling at the wide variety of human pores and skin fibroblasts (HDFs) became investigated. The cells had been incubated with zero. Five, 2.5 and five mM samples for forty-eight hours.

As proven in Figure 1, LApeptide unions inspired fibroblast proliferation interest, and the wide variety of cells elevated with the aid of a most of 24.5% for LAeKTKS and thirteen. Three% for LAePEGekKTKS at Zero.5 mM, respectively. It is likewise critical to becoming aware of the authentic pastime of ECM manufacturing as a way to broaden the cosmetics of the oligopeptide. Ultraviolet (UV) radiation from publicity to daylight is the number one aspect in untimely pores and skin getting old or taking photos. MMP-1 is a chief regulator and performs an outstanding position within the crumble of the outer matrix of the pores and skin, i.e. collagen I and III, all through the system of taking photographs, at the end main to collagen deficiency and wrinkles[18].

Key inhibitors of collagen MMP-1 can be beneficial to anti-getting old retailers. To observe the impact LAeptide is coupled to MMP-1 expression in HDFs, fibroblasts culture have been uncovered to UVA (6.3 J/ cm²) the use of ultraviolet mild Light

that did no longer have an effect on cellular survival. After seventy-two hours of ultraviolet radiation, MMP-1 protein stages had been decided on the center of lifestyle thru an enzyme-related enzyme (ELISA) check. Treatment with LAeptide Comparisons Inhibition of MMP-1 Expression from UVA In a dose-based way, LAePEGekTKTS changed into the handiest inhibitory hobby in opposition to collagenase, blocking off at the least sixty-nine .five% of MMP-1 expression as a result of UVA irradiation at zero. Five mm. LAePEG, LAeKTKS, and LAePEGekKTKS save you the MMP-1 expression from ultraviolet radiation with IC50 values of zero. Eighty-two, zero. Five and zero-Sixteen mm, respectively. The fee of IC50 becomes the compound dose Inhibition of MMP-1 expression because of 50% UV radiation[19]. To compare the anti-getting old sports of LAeptide comparisons, we additionally studied the stimulating impact on collagen synthesis of HDFs. Treatment cells With LAeptide coupled with multiplied collagen manufacturing after which handled with KTKS cells. The consequences confirmed the capability of conjugates to stimulate collagen formation fibromyalgia.

3. Experimental protocols

3.1. Chemistry

The water solubility of leptide aggregates (1e2) became greater than one hundred mg / mL. Cellular toxicity changed into detected from LAeptide comparisons by means of assessing the consequences of mobile survival. The maximum non-poisonous awareness of survival of B16F10 pores and skin most cancers cells, which became the best dose examined and did no longer lessen the validity of a hundred% of B16F10 pores and skin most cancers cells after forty-eight hours, at 37 °C for incubation, (see table 1). The indicated values. The most non-poisonous attention of cellular survival changed into that leptide unions (1e2) have been much less poisonous to B16F10 cancer cells than LA cells. LA confirmed an enormous cytotoxic impact on mobile survival, down To zero.1 mm, even as massive version became discovered within the LAe (1e2) peptide complexes. The impact of dose-established LAeptide coupling at the wide variety of human pores and skin fibroblasts (HDFs) turned into investigate[20].

The cells have been incubated with zero. Five, 2.five and five mm samples for forty-eight hours. As proven in Figure 1, LAPeptide unions inspired fibroblast proliferation interest, and the number of cells accelerated with the aid of a most of 24.five% for LAeKTKS and thirteen. Three% for LAePEGekKTKS at zero.Five mm, respectively.All Fmoc-included amino acids, HBTU, HOBt yet a pair of- chlorotriyl chloride resin (zero. Eighty two mmol/g) have been bought beside GL Biochem about China, whilst every about the special chemical substances bear been offered out of Sigma-Aldrich yet old besides in a similar fashion purification. 1H nuclear magnetic resonance (NMR) spectra have been recorded among CDCI3 yet CD3OD of a JEOL FT/NMR spectrometer at 500 MHz the makes use of on tetramethylsilane (TMS) due to the fact the inside preferred. Chemical shifts are suggested among ppm (d). Reactions had been routinely monitored thru appearing skinny pathway of iodine humidity or UV lamp.

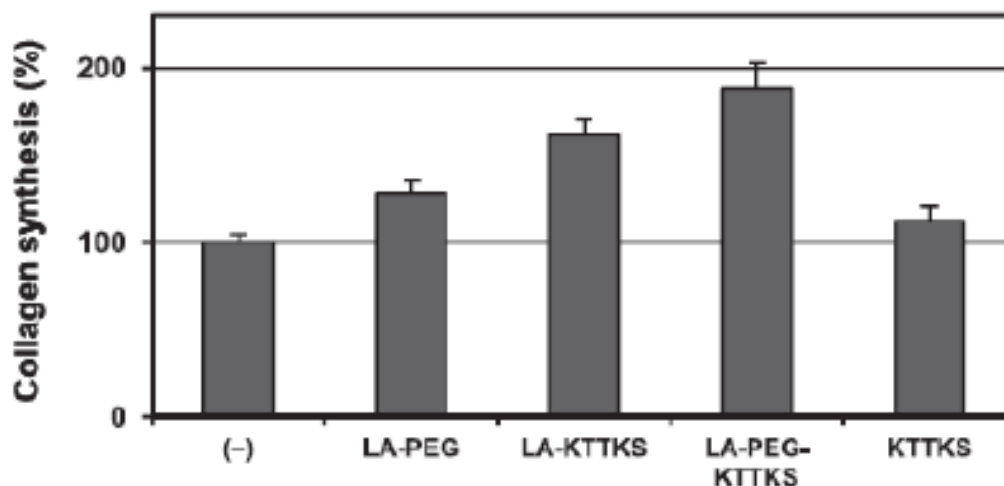


Fig. 2. Influence on LAE1 (type I procollagen) development. The overall collagen content was calculated including the enzyme immunochemical research kits. They have positive-hedonic and negative-cataphorotic qualities.

3.2. Synthesis of LAeptide conjugates

3.2.1. Synthesis of PEGylated LA ester derivative (LAePEG) (4)

Xylene 80 (0.06 perchloro-1,4 chloroethyl-3- (tributylamino)carbimide (EDCI), and 4-dimethylamino-methox-pyridobor-2-2-yl)ethyl-2-oxo-piperidine (DMPO-2,O2) (dimethyl amino) Pyridine (0.032 g, 0.2 mmol) and degassed to provide degassed degassed degassed dichloromethane (15 mL) had been put in a vacuum flask, which was then placed under N₂ for 20 minutes A solution of roxylboxyboxy-PEG (3 mmol) was chilled in an ice bath, then a solution of dichloromethane (15 mL) in ome (60 g, 3 mmol) was applied. The quantity is reduced by 50% in advance, and has been adjusted by apartment temperature, so after it has been stirring for 1 hour:doing away with the residues, to be performed three times on mixed materials:to use an equalize aggregate on the mixture to wipe away all the ingredient goods Segments turned out to be anhydrous/dried over (dry to the point of becoming a solid) and run through a centrifuge to be fully anhydrous and flit (LAePEG) (90-six %) namely a gray passionate with a slight curcuma longa coloration. ¹H NMR (500 MHz, CDCl₃): durabilityd (ppm) 4.21 (t, 2H, J ¼ 5. Zero Hz), 4.19 (t, 2H, J ¼ 4.Five Hz), 3.Sixty-3 (m, w180H, PEG backbone), 3.08e3.22 (2H, m), 2.Seventy (br s, 1H), 2.39e2.47 (m, 1H), 2.33 (t, 2H, J ¼ 7.5 Hz), 1.83e1.90 (m, 1H), 1.57e1.70 (m, 4H), 1.36e 1.50 (m, 2H).

3.2.2. Synthesis of KTTKS-Resin (5)

3.2.4. Synthesis of LAeptide conjugate 2 (LAeKTTKS)

The conjugate 2 was designed and integrated into the LA with the assistance of the system-operated for-LA 1 (340 mg, 59 percent). Competitive metabolite: 2,4 4H NMR (500 MHz,CD₃OD): 4.454 (d, 2H), 3H, 3.86 (d, 2H), 3.31 (m, 4H), 3.27 (m, 0.96H), 2.41 (m, 0.1H), 1.48 (m, 0. (m, 8H).

3.3. Cell subculture

under a resulting in a CO₂ atmosphere in which these stem cells had been maintained at for weeks under Dulbecco's standard (KDM modified eagle stem cells, Gibco BRL, NY, were cultured along with mouse or human dermal fibroblast cells under ten percent fetal bovine serum in a humid setting for months with 1% penicillin-streptomycin and 0.2mM α -mSCF a resulting DM at 7 in a CO₂ rich environment for an additional five weeks.

3.4. Cell viability and proliferation assay

The proliferation and cell viability of MT are tested with the 2, 5-diphenylthiazolium (DTZ) bromide (3, 5-s DT) derivative (MT) Various pretreatment concentrations had been added to the cells prior to their observation. The responses have been inactivated, MTT has emerged after 4-9 hours and the cells have been held at 37C for 3 hours after that time final absorbance eventually showed up on a microplate reader at 570 nm and gained a feasible amount of microtiter plate value of absorbance.

3.5. The assay of Tyrosinase inhibition

By amending Tyrosine with L-DOPA, the approach's ability to differentially absorb light improved dramatically Since there was phosphate-buffered saline (2.0 mM) plus 0.1 mL of mushroom tyrosinase (625 U), the hybrid solution had a "initial" volume" response" of 0.25 mL mushroom tyinase, 0.25 mL of solution, and 10 to 15 min incubation at 25C. To solve the problem, an answer was conceded out by addition 0.03% L-DOPA. Approximately after incubation. The measure between microplate readers was taken at 475 nm after incubation was complete [21].

3.6. Measurement of melanin substance

B16F10 had been completed after seventy-two hours of treatment with the mentioned treatment. Per cell had been count and cleaned with sterile phosphate-buffered saline (PBS) prior to lysis (3000 rpm, 5 m). After the cellwashing, the pellet has remained soluble. Forsome values of [molarity of]H, add 5 mL of Homogenate, for homogeneity's sake (50mM sodium phosphate, pH 6.eight; 1 percent Triton X-one hundred; or a couple over mM PMSF). Melan colour, which can track at 405 nm, otherwise there will be much need of synthetic color (Sigma) [22].

3.7. Enzyme-related immunosorbent assay

Adjusted MMP-1 revealed in the presence of ELISA findings The HDFs were in conformity with the normal water irradiation (at 6.7 J/cm²) When used in conjunction with UVA treatment, the cells have previously been bleached twice with phosphate-buffered saline (PBS). The anti-monoclonal antibody transforms into free to connective tissue and incubated at 37 degrees Celsius for 60 minutes. The pet antibody (conjugated with alkaline phosphatase) remained intact for 30 minutes after being added to the bland PBS Since cleaning, the optical clearness of the ruby ring is almost the same as it was until it was washed[23].

Conclusions

We have designed and synthesized over the LAeptide comparators including anti-melanin or collagen-inspired bio-synthesis outcomes. The conjugates have been immature for cells or discouraged melanin adjustment then hobby of tyrosinase into pores yet skin close cancers cells B16F10. Paired together with 1 and a yoke on inhibits the manifestation on collagenase appropriate after UVA. The bound peptide did in modern times no longer show certain hobby. These effects represent cosmetics and feasible prescript redress strong pores yet skin whitening packages anti-getting historic sellers.

References

1. A Kanchana, S. Devarajan, and S. R. Ayyappan, "Green characterization of palladium nanoparticles and its conjugates trilobatum leaf extract," *Nano-Micro Lett.*, 2014. synthesis and from solanum
2. Q. Hu and Y. Luo, "Polyphenol-chitosan conjugates: Synthesis, characterization, and applications," *Carbohydrate Polymers*. 2016.
3. M. J. Kogan, I. Olmedo, L. Hosta, A. R. Guerrero, L. J. Cruz, and F. Albericio, "Peptides and metallic nanoparticles for biomedical applications," *Nanomedicine*. 2007.
4. S. A. Kazane *et al.*, "Self-assembled antibody multimers through peptide nucleic acid conjugation," *J. Am. Chem. Soc.*, 2013.
5. H. Lee, K. Lee, and G. P. Tae, "Hyaluronic acid-paclitaxel conjugate micelles: Synthesis, characterization, and antitumor activity," *Bioconjug. Chem.*, 2008.
6. Abdullah Hasan Jabbar *et al.*, ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES AGNPS AND ANTIBACTERIAL ACTIVITY, *Turkish Journal of Physiotherapy and Rehabilitation*; 32(3), p. 11225-11231
7. G. Saravanakumar *et al.*, "Hydrotropic hyaluronic acid conjugates: Synthesis, characterization, and implications as a carrier of paclitaxel," *Int. J. Pharm.*, 2010.
8. Y. Chau, F. E. Tan, and R. Langer, "Synthesis and characterization of dextran-peptide-methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase II and matrix metalloproteinase IX," *Bioconjug. Chem.*, 2004.
9. C. Gaillard *et al.*, "Peptide nucleic acid-nanodiamonds: Covalent and stable conjugates for DNA targeting," *RSC Adv.*, 2014.
10. J. J. Castillo *et al.*, "Synthesis and characterization of covalent diphenylalanine nanotube-folic acid conjugates," *J. Nanoparticle Res.*, 2014.
11. S. K. Das, C. Dickinson, F. Lafir, D. F. Brougham, and E. Marsili, "Synthesis, characterization and catalytic activity of gold nanoparticles biosynthesized with *Rhizopus oryzae* protein extract," *Green Chem.*, 2012.
12. S. Dünhaupt, J. Barthelmes, C. C. Thurner, C. Waldner, D. Sakloetsakun, and A. Bernkop-Schnürch, "S-protected thiolated chitosan: Synthesis and invitro characterization," *Carbohydr. Polym.*, 2012.
- A. Pang, C. Li, X. Wang, and J. Hu, "Land use/cover change in response to driving forces of Zoige County, China," in *Procedia Environmental Sciences*, 2010.
13. M. A. Bender, I. Ginis, and Y. Kurihara, "Numerical simulations of tropical cyclone-ocean interaction with a high-resolution coupled model," *J. Geophys. Res.*, 2008.
14. M. Corazza, C. Maranini, S. Bacilieri, and A. Virgili, "Accelerated allergic contact dermatitis to a transcutaneous electrical nerve stimulation device [5]," *Dermatology*. 1999.
15. B. Trisakti, P. Mhardela, T. Husaini, Irvan, and H. Daimon, "Production of oil palm

- empty fruit bunch compost for ornamental plant cultivation,” in *IOP Conference Series: Materials Science and Engineering*, 2018.
16. V. F. and M. M., “An empirical contribution to schizophrenic symptomatology and psychopharmacological processes,” *Ann. Med. Psychol. (Paris)*., 2009.
 17. F. Verhaegen and M. Musiol, “An empirical contribution to schizophrenic symptomatology and psychopharmacological processes,” *Ann. Med. Psychol. (Paris)*., 2009.
 18. J. Hernandez, D. Dubois, R. Marchant, M. Cosca, and J.-B. Edel, “La rotation miocene inferieur du bloc corso-sarde; nouvelles contraintes paleomagnetiques sur la fin du mouvement,” *Bull. la Soc. Geol. Fr.*, 2005.
 19. NCT02625272, “A Novel Technique of HALS With CME and CVL for RCC,” <https://clinicaltrials.gov/show/nct02625272>, 2015.
 20. Y. Purwamargapratala, S. Sudaryanto, E. Kartini, and M. Manawan, “Synthesis of $\text{Li}_4\text{Ti}_5\text{O}_{12}\text{-Sn}$ by ultrasonic method as anode materials for lithium ion battery,” in *IOP Conference Series: Materials Science and Engineering*, 2018.
 21. H. Yoshida, Y. Kobayashi, Y. Mita, and H. Miyashiro, “Quantitative Evaluation of Charge-Discharge Cycle Test and Storage Test in Commercially Available Lithium-Ion Batteries,” *Meet. Abstr.*, 2015.
 22. J. Ryan, “Report: 2001 Anthrax Attacks Were Preventable,” *ABC News Online Edition*, 2011.