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Biomedical Evaluation of Chitosan-Gelatin Transdermal Patch Embedded With Bio-Silver Nanoparticles as A Wound Dressing Material:-An *In Vitro* Study

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Abstract: Chitosan prepared from natural polymer chitin and cast into transdermal patch with bio-silver nanoparticles isolated from *Ganoderma lucidum* as a drug has been tested for the wound healing activity. Chitosan prepared with 85% degree of deactyelyation and confirmed by FTIR (Fourier transform Infrared spectroscopy) and XRD (Xray diffraction spectroscopy);*Ganoderma lucidum* used to synthesis silver nanoparticles and confirmation was done by TEM (Transmission electron microscope),EDAX (Energy dispersion xray spectroscopy) and XRD (Xray diffraction spectroscopy).An attempt has been made here to formulate transdermal patches.,Chitosan was added for the flexibility and permeation enchancer,Glycerol was used as plasticizer, Gelatin to give the moisturizing content and silver nanoparticles acts as cross linking agent.The prepared transdermal patch were then evaluated for biomedical parameters such as bioadhesion strength, heamocompability,*in vitro* degradeation and microbial penetration test. The results showed by transdermal patch could meet the essential requirements for reasonable wound dressing material with desirable characterstics and fulfilled the requirements to obtain the transdermal patch for wound management.
Keywords: Chitosan,Gelatin,Silvernanoparticles, Ganoderma lucidum,scratchassay,wound healing.

Introduction:

Drugs products that are dermally administered through the skin falls into two categories: those applied for local action and systemic effects. Local actions effect include those applied on or at the surface of the skin,which exerts the action on the stratum corneum or modualating the function on epidermis or dermis. The products that come in this criteria arecreams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols and solutions. Transdermal drug delivery systems (TDS) or transdermal patches come under the criteria of systemic effect[1].

Transdermal drug delivery system represent delivering of self contained, discrete dosage form of drug to the general circulation through the skin.It overcomes the oral drug delivery system and hovering to provide an alternative solution for the exertion, Moreover it terminates gastro intestinal toxicity and heptatotoxicity[2].Unlike the oral dosage forms which produce blood hikes and troughs, transdermal patch provides uniform dispersion of drug into target side .The bio-adhesive skin patches to deliver drugs systematically a new phenomenon[3].The application of the theraupetical to the skin to ease the ailments has been followed since the time of ages and has showed an beneficial outcomes.

Wound dressing material, targets to various stages of wound healing and create better healing envionment. It protects the wound surface to boost up the healing process. Unique awareness has been paid to biopolymer transdermal patch with novel properties such as immediate pain control, easy replacement, transparency used to record the healing process, absorb and prevent loss of body fluids, barrier against bacteria, oxygen permeability, good handing and direct the release of drug dosage[4,5,6].

The advancement in the field of polymer science has laid a path towards the transdermal delivery systems with the considerable flexibility using natural,synthetic and semisynthetic polymers to stabilize the release of medicine via the intact skin.Thus the polymer acting as backbone in the transdermal delivery[7,8,9,10].

Chitosan and gelatin are natural polymer that are non toxic, biocompatible and biodegradable. Chitosan posses lot of properties like drug delivery, cell delivery systems, orthopaedics, wound healing, ophthalmology, bone healing, anti-microbial, heamostastic etc[12-17],Gelatin, aprotien derived from collagen and bones are translucent, colourless, odourless, brittle and tasteless. It has a very good film forming property and known for wound healing properties[18,-,22], .Bio-Silver nanoparticles synthesized from Ganoderma lucidum posses lot of beneficial properties; Anti-microbial, anti-diabetic, Wound healing, anti-inflammatory and angiogenesis properties.The drug that are in nanosize help in easy transferred and targeted to specific site[23,24,25,262,27].

Since Chitosan, gelatine and bio-silver nanoparticles synthesized from Ganoderma lucidum are having good wound healing and anti-microbial activity, it is anticipated the combination effect of these biopolymer with nanosized drug (Bio-Silver nanoparticles) compounds results may serve as promising film forming matrix for transdermal delivery of drugs into skin.

Materials and method:

Gelatin and Silver nitrate were purchased from HIMEDIA Laboratories Pvt. Ltd; glycerol, Sodium hydroxide, calcium chloride, sodium chloride, nutrient broth, Hydrochloric acid were bought from Fisher scientific, THERMO ELECTRON LLS INDIA Pvt. Ltd.

Chitosan Preparation:

The collection, isolation, extraction and characterization of chitosan molecule was done and published at [28] have been taken for further analysis.

Biosynthesis of Silver Nanoparticles:

35mg of silver nitrate was added to 250ml of water. The solution was uniformly mixed .To obtain silver colloids 10 ml of the mushroom extract(20gms Ganoderma lucidum and 100ml of deionised water) and 90 ml of the silver nitrate solution was added and incubated for one hour at room temperature (25℃). The detection of silver nanoparticles was confirmed by the appearance of light yellow colour to dark brown on the complete reduction. The pH of the solution was adjusted to around 7.5. The sample was read under UV visible spectrometer for the presence of silver nanoparticles and characterization of silver nanoparticles was done by various analytical techniques such as TEM, EDAX and XRD[29,30].

Preparation of Chitosan–Gelatin Transdermal Film:

A series of chitosan–gelatin composite films were prepared by varying the ratio of constituents[31]. The values obtained were then optimized.

Table 1: Chitosan–Gelatin Transdermal Film with varying conc.silver nanoparticles

Composition	TDF1	TDF2
CHITOSAN	0.5g	0.5g
GLYCEROL	400 µl	400 µl
GELATIN	2.0g	2.0g
SILVER NPs	400 µl	800 µl
WATER	30	30

Characterization of dermal patches[32]

Contact angle measurement:

To evaluate the hydrophilicity of Static contact angle of distilled water on the transdermal patch by manually a contact angle meter.The dermal patches from six different places were cut into 2 cm X 2 cm measured and results are averged.

Bio adhesive strength measurements :

The bioadhesive strength was determined by previously published method[33] using hairless chicken skin. A circular pice of transdermal film was cut and holded with an adhesive tapeon the ground surface of a tissue holder made of plexiglass and the film was glued to another holder of same size. It moistened with 25µl of phosphate buffer pH 7.4.The two holders were in contact with each other with uniform and constant pressure for 5 min. The tissue holder was allowed to hang on an iron rod with the aluminium wire an pre weighed polypropylene bag was attached to the hook on the backside of the film holder with a piece of aluminium wire. After 5 min, water was added to the polypropylene through intravenous infusion by constant duration of time and time noted for deattachment of the film from skin was noted.

Haemocompatibility study

Thrombus formation test :

Six transdermal patches were cut into 2 cm X 2 cm from each batch. The patches wer immersed in normal saline for 24 hours at 37°C.After the patch has been swelled, 0.5ml of ACD blood, 0.03% Cacl(4 M) to start the thrombus formation, distilled water has been used to stop the reaction. Then it was fixed with 36% formaldehyde after 10min washed with distilled water and air dried and weighed. The same procedure was repeated for the glass surface for control and the respective weight of thrombus formed was measured.

Haemolysis assay:

Six transdermal patches were cut into 2 cm X 2 cm from each batch. The patches were immersed in 10 ml of saline at 37 ± 2°C for 30 min. Then 0.2ml of ACD blood was added to test tube and incubated for 60 min. For positive control 0.2 ml of ACD blood and 0.5 ml of HCl(0.01N),negative control 0.2 ml of ACD blood with 10 ml saline was incubated for 60 min. The optical density was measured after the centrifugation at 545nm.

The percentage haemolysis was calculated from the following equation

Haemolysis (%) =

Atest sample-A negative control/Apositive control-A negative control X 100

Microbial penetration study :

The ability of transdermal patches to prevent microbial penetration was tested by placing the patches as cap at the mouth of the vial containing 5ml of nutrient broth. In positive control, the vials are kept open and negative control, vials are capped by cotton plug. It left undisturbed for 7 days and the cloudiness was noted as microbial contamination.

Oxygen penetration measurement:

Randomly six different transdermal patchs were taken and place on the top surface of the water 200ml in the 250 ml flask and held in screw lid. The negative control was the closed flask with air tight cap, while positive control was the open flask. After 24hrs incubation, measure the dissolved oxygen with winkler method.

In vitro degradation test:

In vitro degradation of the transdermal patches was investigated using 40 ml phosphate buffered solution (pH 7.4) containing 1.6 µg/ml human serum. The six transdermal patches from each batch cut into 1 cm X 1 cm were placed in phosphate buffer containing serum solution in shaking water bath at 37 ± 0.5°C for 2 hours. The transdermal patch was washed with distilled water and oven dried at 80 ± 0.5°C.Then the weight loss of transdermal patch were measured:

Remaining weight (%) =(Wd/Wo) X 100

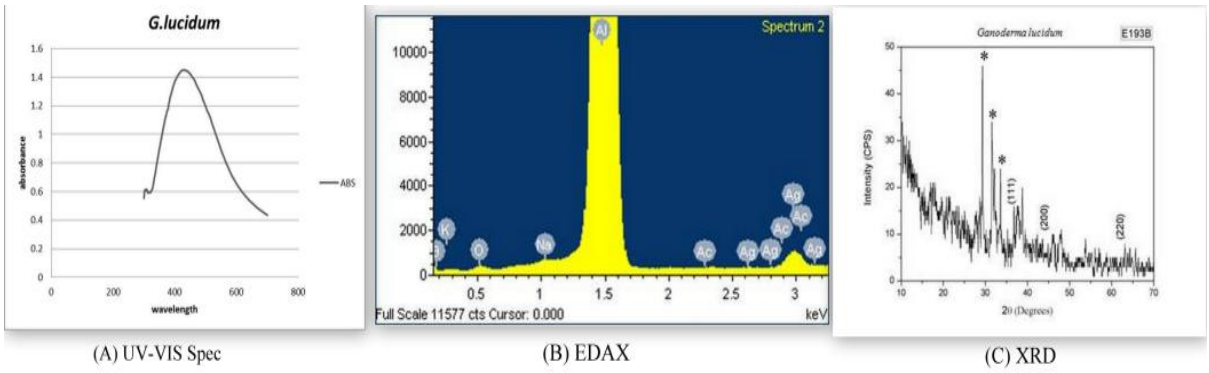
Dispersion characteristics/wet integrity test:

The transdermal patches (5 cm X 5 cm) were cut and added to 50 ml phosphate buffer (7.4pH) in 250 ml flask. The flask is gently swirled for 1 min without causing a vortex and the integrity of the dermal patch was visually established.

Results and discussion:

In this work chitosan-gelatin based transdermal film incorporated with Bio-SNPs as a novel wound dressing material, which were prepared by casting and evaporation method. Bioevaluation of transdermal film have shown different characterstic feature according to different composition of silver nanoparticles. The synthesis of silver nanoparticles from ganoderma lucidum was confirmed and characterized by UV-Vis Spectroscopy, FESEM, EDAX and XRD, shown in fig 1-3.

Fig1 :Ganodermalucidum



(A) UV-VIS Spec (B) EDAX (C) XRD

Fig 2:(A)UV-Vis spectrum after 30 mins of incubation(B) EDAX analysis of *G.lucidum* AgNPs (C) XRD analysis of *G.lucidum* AgNPs

Contact angle of transdermal film changes along with time rention of the film to swell by dropping the 1 drop of water onto the film from 0-30 sec, the contact angle of TDF1-58.4 ± 1.01°, TDF 2- 43 ± 0.3° and C1 27.4 ± 1.32°at 30 sec for formulation of transdermal film respectively. The swelling rate of the film was very fat, after certain particular time; the contact angle started to gradually decrease due to strong water solubility of transdermal film. After 3-4 minutes the water was completely observed, the contact angle changed 0-180°.The swelling increase due to the combination chitosan-gelatin-bio-silver nanoparticles, when compared with chitosan film, as it's hydrophilic and brittle in nature. The lower the contact angle posses higher hydrophilicity and the higher contact angle lower hydrophilicity.

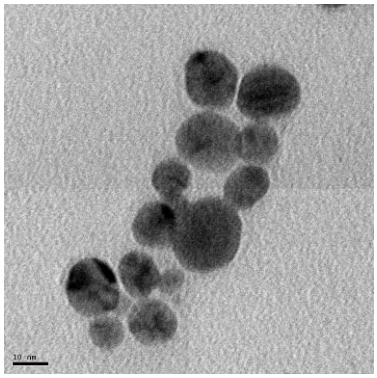


Fig 3:HR-TEM-silver nanoparticles

Bioadhesive properties of transdermal film were observed and shown in table 2. Chitosan-gelatin transdermal film gave the best adhesive results and glycerol also good plasticizer. Thus showing more soft and elasticity nature.[34]

Table 2: Physiochemical properties of chitosan-gelatin transdermal film embedded silver nanoparticles

	Contact angle	**Thromo blytic (g)**	**Heamolysis (%)**	**Oxygen penetration (mg)**	**Microbial penetration**	**In vitro degradation (%)**	**Bio-adhesion (g)**
Positive control	27.4±0.32º 27.4±0.32º	0.09±0.32	-	9.6±1.1	Turbity	-	
Negative control			-	5.32±1.1	-	-	
TDF 1	58.4±1.01º 65.4±0.22º	0.162±0.30	5.23±1.1	8..323±0.41	-	82	10.6 ± 0.9
TDF 2	43.0±1.32º 56.54±0.32º	0.062±0.04	9.8±0.82	7.32±0.052	-	86	12.11± 0.09

Heamocompatibility test was proved by all transdermal patches to achieve the good wound dressing material. The antithrombogenic properties of the patches were done and found to be at the range of 0.162 ± 0.002g, 0.06 ± 0.04g and the percentage heamolysis assay determined to be 5.23 ± 1.1 % and 9.8 ± 0.82 % for he samples TDF1 and TDF2 patches respectively(table 2).From the results obtained.clots formed was low and percentage of heamolysis was comparability low and could be considered as best wound dressing material holding the heamocompatible properties.

The oxygen penetration properties of the transdermal patches. The dissolved oxygen content mean value of the positive control, negative control,TDF1 and TDF2 was found to be 9.6± 1.21mg/ml,5.32 ± 0.20 mg/ml , 8.5 ±0.41mg/ml and 7.3 ± 0.052mg/ml.The results obtained using the patches almost ive the similar results.Thus showing the oxygen transfer through the patches, helping in speedy recovery of wound.[35]

Microbial penetration test was followed to check the microbial penetration through the patches towards the wounds site results in slow and infection in wound healing mechanism. The microbial contamination was

not seen any of the tubes with dermal patches and the negative tubes, positive tube alone was turbit after 7 days of incubation. This indicates the developed patches has the ability to protect the wound bacteria infection.[35]

In vitro degradation of transdermal patches were found to be degradable at the percentage range of 80-90%.This indicates the prepared product is an ecofriendly product with a good stability.

To determine the original structure of transdermal film is retained,wet integrity test is followed and transdermal film with no dispersion retain its orginalform. Thus to remove easier from wound area.

Conclusion:

The biomedical evaluation of chitosan-gelatin transdermal film with bio-silver nanoparticles had meet the basic requirement for a wound dressing material, In vitro studies of the patches gave or achieved their targets giving the preeminent results less contact angle, good bioadhesion, significant heamocompatilibity, exceptional barrierity against microbial penetration, slow but complete degradation and mechanical properties.

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