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PREPARATION AND EVALUATION OF NANOGEL CONTAINING ESTRADOL LOADED NANOPARTICLE FOR BONE REGENERATION

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ABSTRACT

Objective:

Nanogel in drug delivery is known to improve premeability, compactable use, and eventually applied on sepcific site where the drug is going to targeted. This study was carried out to show the estradiol nanogel for the potential treatment in bone regeneration and to evaluate the effectiveness in vitro and models.

Methods: Nanogel of estradiol was prepared with PLGA and Pluronic F68 by the process of modified nanoprecipitation technique with different concentrations of PLGA. The particle size, zeta potential, scanning electron microscopy (SEM), and in vitro drug release were performed.

Result: The nanoformulation shows least particle size of 151 nm and maximum zeta potential value of 33.8 mv with 88.57% entrapment efficiency were observed with EP3 formulation. The SEM and in vitro drug release of EP3 were showed shape with controlled release when compared to other formulations.

Conclusion: This study concluded that our nanogel shows a significant advantage in bone regeneration in vitro

Keywords: Estradiol, PLGA, Pluronic F68 , Nanoprecipitation, Boneregenration

INTRODUCTION

Bone is the only body tissue capable of regeneration, allowing the restitution-adintegrum following trauma. In the event of a fracture or bone graft, new bone is formed, which following the remodeling process is identical to the pre-existing. Bone is a dynamic tissue in constant formation and resorption. This balanced phenomenon, known as the remodeling process, allows the renovation of 5-15% of the total bone mass per year under normal conditions **[1]**. Bone remodeling consists of the resorption of a certain amount of bone by osteoclasts, likewise the formation of osteoid matrix by osteoblasts, and its subsequent mineralization. This phenomenon occurs in small areas of the cortical bone or the trabecular surface, called "Basic Multicellular Units" (BMU). Nanogels may be defined as highly cross linked nano-sized hydrogel systems that are either co-polymerized or monomers which can be ionic or non-ionic **[2, 3]**. The size of nanogels ranges from 20-200 nm **[4]**. They can escape renal clearance and prolonged serum half-life period due to their size. Nanogels are three dimensional hydrophilic networks that have the tendency to imbibe water or physiological fluid in a large amount, without changing in the internal network structure. Biodegradable polymers such as methoxy polyethyleneglycol-poly (caprolactone) (MPEG-PCL), poly lactic-co-glycolic acid,poly lactic acid, chitosan, gelatin, polycaprolactone, PLGA, PLA and poly-alkyl-cyanoacrylales are used for nanoformulations **[5, 6]**. Poly (lactic-co-glycolic acid) is one of the best characterized biodegradable copolymers that decomposes to non-toxic products PLGA have high hydrophobicity, which leads to lower degradation and thus slower drug release rate **[7-9]**.

We previously demonstrated that Bone regeneration NPs can available only on transdermal patches in the drug delivery administration Estradiol is a well-known hormonal drug which mainly used postmenopause women and also used in bone regeneration too which does not producetoxicity as a side effect with the frequent administration in dose regimenminimal amount of the drug. However, loading Estradiol NPs and their transport to the bone memberane in an active form and sufficient concentrations remained a challenge. In this study, we have the first developed the conditions to load Estradiol to NPs and then performed the characterization, entrapment efficiency and in vitro drug

release. And then the nanosuspension was incorporated into the gel. Hence, this study is carried out to enhance the targeted drug deliveryof Estradiol in combination with polymers for thebone regeneration.

METHODS

Estradiol, PLGA and Pluronic F 68 were purchased from Sigma-Aldrich, Bengaluru, India. All the other chemicals were used as an analytical grade.

ATUTNATED TRANSMISSION INFRARED ANALYSIS:

Atutnated Transmission Infrared Analysis was carried out for the estradiol and plga to find out the functional group and to find out whether any incomptability is present

Preparation of Esradiol nanosuspension

The preparation of Estradiol nanosuspension is carried by modified nanoprecipitation method **[8, 9]**. In this method,phosphate buffer with pH5.0 was used as an external medium instead of aqueous phase. Various concentrations ranging 10-50 mg of PLGA and 10 mg Estradiol were accurately weighed and dissolved in 5 ml acetone. This organic solution was added slowly to pluronic F 68 (1%) in phosphate buffer (pH5.0) solution. The organic solvent was then allowed to evaporate for 2 hrs with continuous stirring on a magnetic stirrer (Remi). The NP suspension was then centrifuged at 15,000 rpm for 30 minutes at 4°C using high-speed centrifuge (Remi). The supernatant was taken for further evaluation.

NANOPARTICLE ANALYSIS

Particle size and zeta potential

The size of the prepared NPs was analyzed using Malvern apparatus. All samples were diluted with ultra-purified water, and the analysis was performed at a scattering angle of 90° and at a temperature of 25°C. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate. The zeta potential is determined using a zeta seizer. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted.

Scanning electron microscopy (SEM)

The surface morphology of the NP suspension was studied using SEM quanta 200 FEG scanning electron microscope (FEI Quanta FEG 200) set at 200 kV by placing an air dried NP suspension on copper electron microscopy grids, and the image was captured at desired magnification.

Drug content

Drug content was determined by taking 1 ml of the PLGA loaded Estradiol nanosuspension. To this formulation 1 ml of

aqueous potassium dihydrogen phosphate solution (30 mM) was added and the mixture was centrifuged at 10,000 rpm at 15°C. The clear supernatant was removed and analyzed by spectrophotomertically, also drug content where calculated.

Drug entrapment efficiency

The drug loaded NPs are centrifuged at 13,000 rpm for 30 minutes and the supernatant is assayed for non-bound drug concentration by spectrophotometer

***In vitro* release studies**

In vitro release studies were performed using diffusion apparatus United States Pharmacopeia-II at 50 rpm 10 ml of the nanoformulation was placed in dialysis membrane having molecular weight cut-off from 12,000 to 14,000 daltons. The membrane was soaked in phosphate buffer saline (PBS) for 12 hrs before using Estradiol nanoparticle formulation in dialysis membrane was placed in the bowl containing 100ml of PBS pH7.4 at fixed time intervals, 1 ml of the aliquot was withdrawn and fresh PBS pH 7.4 was replaced to maintain constant volume.

Preparation of crosslinked carbapol hydrogel:

1.5 g of carbapol were dissolved in 20.0 mL of 2.0% aqueous acetic acid in a beaker at room temperature with continuous soaking for 24 hours to obtain pale yellow viscous carbapol solutions. Few drops of 0.5% tween-80 were again added to the solutions. The solutions were also filtered with the sintered glass crucible and 0.1% aqueous glutaraldehyde solution in different amounts. The solutions were stirred for 30 minutes at room temperature as they became increasingly viscous and with more intense colour. These solutions were thereafter cast into a petri dishes and dried overnight at room temperature to form the crosslinked carbapol hydrogels. The semi-dried, crosslinked hydrogels were further dried in an oven at 45°C for 12 hours to completely remove the residual solvent

INCORPORATION OF NANOSUSPENSION INTO GEL BASE TO FORM NANOGEL

Approximately 3.8 mg of Nano suspension was taken and incorporated into gel to form nanogel

RESULT

Atutnated Transmission Infrared Analysis

Atutnated Transmission Infrared Analysis was carried out for the estradiol and plga to find out the functional group. The figures of Drug, Polymer and Drug + Polymer was given below. FTIR analysis result for estradiol shown the value in the starting of peak is 3438.78cm^{-1} and the highest value in

the peak is 2936.21cm^{-1}.The FTIR analysis result for PLGA polymer shows the value is the starting peak of 2948.49cm^{-1} and the highest value in the peak are 1747.69cm^{-1} .And the final analysis result of drug and polymer shows the value for stating peak is 3438.78cm^{-1} and the highest peak of 2960.45cm^{-1}. This shows the drug and polymer doesn't have any incompatability.

Preparation of Estradiol Nano suspension

The preparation of Estradiol Nano suspension is carried out by modified Nano precipitation method and the formula for the Estradiol Nano suspension is given in **Table 1**.

Particle size and zeta potential

The particle sizes were shown in **Table 2** which describes the increase in polymer concentration having an impact on particle size. The Estradiol loaded PLGA Nano formulations of 1:3 shows 151 nm in the table may be chosen as better particle size because another ratio of 1:1,1:2 having less polymer so that more drug may not be encapsulated. On the other hand, 1:4, 1:5 are having more amount of polymer with high zeta potential, hence the 1:3 ratio with 151 nm particle size with 33.8 mv zeta is chosen due to the higher value of zeta potential implies more stable. The size and stability may be compromised to achieve a better result.

Scanning electron Microscopy:

The result of scanning electron Microscopy analysis, **Figure 4** shows the smooth in surface, spherical in shape and seen to be good.

Drug content and entrapment efficiency

The drug content of the prepared Nano formulations were determined and the results shows 3.98 mg/ml with 88.57% entrapment efficiency in with EP3 higher drug content having more entrapment efficiency which was shown in **Table 3** since other formulation shows low drugs content when compare to EP3 with varying entrapment efficiency. Thus, based on result EP3 may be best formulation among the remaining Nano suspension.

***In vitro* release studies**

Table 4 shows *in vitro* drug release of all formulations; in that, EP1 and EP2 shows the lowest drug release but was rejected as the particle size was very lowEP4, EP5, were not selected despite low particle size because they act as release retardants due to a high concentration of polymer. Hence, EP3 is selected as the best formulation due to its optimum drug release 96.53 in 24 hrs which is an controlled release.

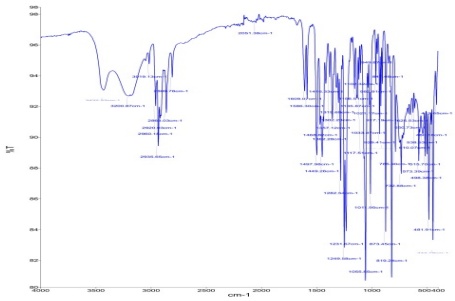

Figure 1: FTIR analysis of Drug

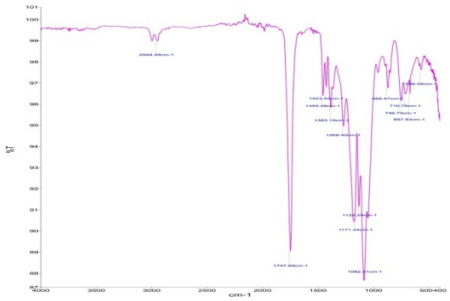

Figure 2: FTIR analysis of Polymers

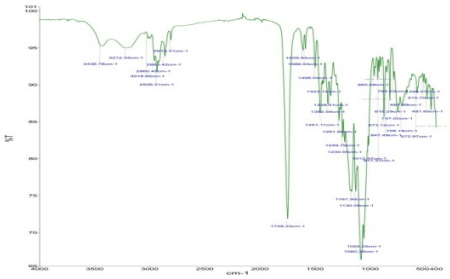

Figure 3: FTIR analysis of Drug and polymer

Table 1: The preparation of Estradiol Nano suspension

Ingredients	EP1	EP2	EP3	EP4	EP5
Estradiol mg	10	10	10	10	10
PLGA	10	20	30	40	50
Pluronic F 68 %	1	1	1	1	1
Acetone ml	5	5	5	5	5

PLGA: Poly lactic co glycolide acid

Table 2: The Particle size and zeta potential of Estradiol Nano suspension

FORMULATION	RATIO	PARTICLE SIZE nm	ZETA POTENTIAL mv
EP1	1:1	130±2.5	33.2±2.7
EP2	1:2	141±1.6	33.4±1.1
EP3	1:3	151±2.3	33.8±2.2
EP4	1:4	175±4.2	33.6±1.5
EP5	1:5	214±1.8	33.1±3.3

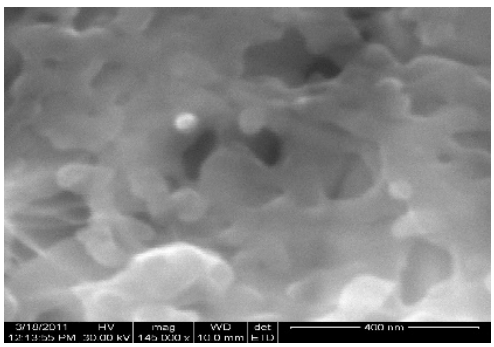

Figure 4: SEM of Estradiol Nano suspension

Table 3: The average drug content and average drug entrapment efficacy of Estradiol Nano suspension

FORMULATION	RATIO	AVERAGE DRUG CONTENT mg/ml	AVERAGE ENTRAPMENT EFFICACY %
EP1	1:1	2.2±0.02	65.14±1.2
EP2	1:2	3.5±0.08	85.29±1.9
EP3	1:3	3.98±0.19	88.57±2.4
EP4	1:4	2.9±0.58	79.32±1.4
EP5	1:5	3.1±0.15	82.1±1.9

Table 4: *In vitro* drug release of all Estradiol Nano suspension

S.NO	TIME hr	% CUMULATIVE DRUG RELEASE				
		EP1	EP2	EP3	EP4	EP5
1	0	0	0	0	0	0
2	1	5.87±1.0	6.32±1.2	7.12±0.3	5.13±0.2	4.15±0.4
3	2	10.42±1.3	12.62±1.3	11.93±1.2	9.45±1.4	10.25±1.4
4	4	19.38±1.1	24.66±1.1	25.41±1.6	21.52±1.6	15.65±2.4
5	6	25.22±1.2	36.51±1.2	38.63±1.5	37.25±1.8	31.85±2.4
6	8	30.27±1.2	45.66±1.4	43.85±0.5	41.52±1.3	45.65±2.4
7	12	41.35±1.3	52.15±1.3	62.11±1.2	59.63±0.6	52.98±1.6
8	16	48.32±1.4	60.15±1.1	69.82±1.0	65.24±1.1	62.26±1.2
9	20	52.44±1.3	64.47±1.3	87.89±1.2	79.74±1.4	71.15±1.4
10	24	58.31±1.4	68.99±1.2	96.53±1.3	85.25±1.2	8.24±1.1

DISCUSSION

By preparing the nanogel for the bone regeneration we found, In the basic identification of FTIR analysis we found in result of drug + polymer the value for starting peak is 3438.78cm^{-1} and the highest peak of 2960.45^{-1}. This value has be 3487.78cm $^{-1}$ identified in the drug and 2960.45cm^{-1}identied in polymer. Here this proved drug and polymer does not have any incompatability. In Scanning Electron Microscopy, the particle was found to be smooth in surface and spherical in shape. In drug entrapment efficacy and in *in vitro* studies it was found that EP3 formulation shows good satisfactory result when compared to EP1, EP2, EP4, EP5 and the drug content is also more in when compare to other formulation. From overall project assessment we found that estradiol + PLGA combination of nanosuspension of accurate weight of 10mg estradiol and 30mg of PLGA is suitable for formulation. And 3.8mg of nanosuspension accurately 2ml of nanosuspension was taken and incorporated into gel form nanogel.

CONCLUSION

This study concluded that our nanogel shows a significant advantage in bone regeneration *in vitro*.

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