Preparation and characterization of PLGA-based biocompatible nanoparticles for sustained delivery of growth factor for wound healing applications

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Platelet-derived growth factor-BB (PDGF-BB) loaded PLGA : poloxamer blend nanoparticles (PLGF) were studied for their physical, chemical and biological characteristics using *in vitro* and *in vivo* experimental models. The particle size of PLGF was between 100 and 150 nm with 85% encapsulation efficiency. *In vitro* released kinetics revealed a slow and sustained release of growth factor (GF) for a month. Furthermore, biological studies confirmed the non-toxic, and non-irritant nature of nanoparticles and angiogenesis stimulating ability of GF released from nanoparticles demonstrating their potential for the treatment of chronic wounds in future.

Keywords: PDGF-BB, PLGA, angiogenesis, wound healing.

STIMULATION of angiogenesis is a critical requirement for treatment of chronic wounds. Under normal conditions, wound healing proceeds in a timely and sequential manner through the inflammatory phase, granulation, contraction, and re-epithelialization of proliferative phase and ends with tissue remodelling[1,2]. Interruption in any of these phases results in the loss of functional and anatomical integrity of the wound, making it chronic to heal. Despite varied etiologies of chronic wounds of diabetes, pressure and venus ulcers, the three factors reported to be common for these wounds are: cellular and systemic effects of ageing, repeated ischaemia-reperfusion injury and bacterial contamination resulting in an inflammatory response[2,3]. A recent study revealed that chronic leg ulcers (CLU) are the common cause of morbidity with prevalence ranging from 1.9% to 13.1% (ref. 4). Such an increase in prevalence and morbidity is primarily due to the poor response of the wound to treatment. Although there are many theories defining the etiology of non-healing chronic wounds, reports suggest the diminished availability of growth factors (epidermal growth factor (EGF), Keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF)), decreased keratinocyte migration, increased reactive oxygen species (ROS), increased activity of tissue proteases and microbial colonization are the main factors for delay in healing process[5]. This in turn lengthens the hospital stay, thereby posing economic burden to patients.

To overcome the limitations of current treatment modalities and to improve the healing process, GFs are being studied largely due to their key role in forming new capillaries for granulating tissue[6,7]. However, until now, clinical trials have been successfully completed only for PDGF-BB and it has been approved by FDA in the trade name Regranex® for the improved healing of chronic neuropathic and diabetic cutaneous ulcers[8]. But the major disadvantages associated with GFs are their short half-life and susceptibility to degradation by proteinases[9] being easily removed by exudates before reaching the wound bed[10]. Hence, in the present study, an attempt is made to develop an appropriate delivery mode using a method described by Csaba *et al.*[11] and studied its ability of stimulating angiogenesis and wound healing through *in vitro* and *in vivo* models.

Materials and methods

Materials

Polylactic co-glycolic acid (PLGA; MW: 30 kDa to 60 kDa, copolymer ratio 50 : 50) and PDGF-BB and its relative ELISA kit were purchased from Sigma, USA. Poloxamer 188 (pluronic F68), bovine serum albumin (BSA), heparin sodium salt (Hp) and other tissue culture consumables such as fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), antibiotics, etc. were purchased from HiMedia (Mumbai, India). L929 cell line (NCTC clone 929) was purchased from ATCC, USA. All other reagents and chemicals used in the study were of analytical grade. Ultrapure water was used throughout the study.

Fabrication of PDGF-BB loaded PLGA : poloxamer blend nanoparticles

PDGF-BB loaded PLGA : poloxamer blend nanoparticles (PLGF) were prepared as described by a modified solvent

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diffusion technique[11]. An aqueous solution of 100 μl containing 2 μg of PDGF-BB was briefly emulsified by vortex mixing with 2 ml of PLGA (2% w/v) and poloxamer (2% w/v) solution in dichloromethane. The resulting emulsion was quickly added to 12.5 ml of ethanol, leading to an immediate precipitation of polymer in the form of nanoparticles (NPs). This suspension was immediately diluted with 12.5 ml of ultrapure water and stirred at room temperature for 10 min. Further, the organic solvents were eliminated through evaporation under vacuum at 30°C using Rotavapor. Finally, the NP suspension was isolated by centrifugation at 8000 rcf for 1 h at 5°C. The NP pellet was resuspended in ultrapure water. To optimize the PDGF-BB encapsulation, Hp (2 μg) and BSA (200 μg) were added as stabilizing agents and included in the 100 μl inner water phase during the preparation step.

For wound healing studies, NPs were encapsulated with different concentrations of PDGF-BB (0.3, 0.4, 0.5, 0.6, 1 and 2 μg) during emulsification process and their ability to stimulate angiogenesis was evaluated.

Optimization and characterization of growth factor loaded PLGA : poloxamer blend NPs

Growth factors (GF) were used at different concentrations during encapsulation, and NPs were characterized to ascertain their reproducibility for *in vitro* and *in vivo* experiments. To determine the optimal formulation parameters with respect to the formulation of GF-loaded PLGA NPs, PLGF, percentage yield, percentage entrapment efficiency, particle size, zeta potential and surface morphology of NPs were analysed.

Percentage yield: NPs were freeze-dried and weighed and the percent yield was calculated using the formula[12]

$$\%\text{yield} = \frac{\text{Weight of nanoparticles obtained}}{\text{Weight of growth factor and polymer}} \times 100.$$

Encapsulation efficiency: Encapsulation efficiency of NPs was determined by alkaline hydrolysis assay. Here, 1 mg of PDGF-BB encapsulated NPs was treated with 1 ml of 0.05 N NaOH under moderate magnetic stirring for 1 h at room temperature. After achieving complete degradation, samples were opportunely diluted and analysed by ELISA. Colour development was monitored with an ELISA plate reader (Thermo Fisher Scientific, China) at 405 nm, with wavelength correction set at 650 nm.

Determination of physicochemical properties: NPs were characterized for their particle size, poly-dispersity index and zeta potential using photon correlation spectroscopy (PCS) and laser doppler anemometry (Zetasizer, Malvern Instruments, UK)[13]. The morphology of NPs was analysed by field emission scanning electron microscope (FeSEM) (Sigma Zeiss, UK).

In vitro GF release kinetics from PLGA : poloxamer blend NPs

GF release kinetics was studied by both direct method and indirect method, i.e. by measuring the amount of GF that remained encapsulated in the NPs[13]. In case of indirect method, NPs encapsulated with different concentrations of GF were incubated in phosphate buffered saline (PBS) (pH 7.4) at 37°C under static conditions. At fixed time intervals, released samples were collected, centrifuged at 8000 rcf for 1 h at 5°C. Then the isolated NPs were degraded with 0.05 N NaOH under moderate magnetic stirring for 1 h at room temperature to achieve complete polymer degradation. Further, the alkaline GF solutions were appropriately diluted and analysed by ELISA to quantify the amount of GF which was not released. The amount of released GF was calculated from the difference between the total amount of encapsulated GF and the amount of non-released GF for each time interval. In direct method, GF encapsulated NPs were treated on L929 mouse fibroblast cells and the release of GF in both media and inside the cells was studied by ELISA using supernatant and cellular extract.

In vitro cell proliferation assay

For *in vitro* cell proliferation, fibroblast cells (passage number 18) were treated with different concentrations of GF encapsulated NPs for the periods of 2, 4, 6, 8, 10, 12 and 24 h. The treatment groups of the study were as follows: group 1: control, group 2: PLGA : poloxamer blend NPs (1 mg/ml), groups 3–8: NPs loaded with 0.3, 0.4, 0.5, 0.6, 10 and 2 μg/ml of PDGF-BB. GF encapsulated NPs were used at the concentration of 1 mg/ml. Five hours before the completion of treatment, the cells were incubated with MTT (10 mg/ml) and the resulting formazon crystals quantified using a photometer at 570 nm (Thermo Scientific, RS-232C, Germany). Cell viability was calculated as

$$\text{Cell viability} = (\text{OD}_{570}\ \text{treated}/\text{OD}_{570}\ \text{control}) \times 100.$$

where OD is the optical density.

In vitro wound healing assay

L929 mouse fibroblast cells were grown in 24-well plates at a density of 1×10^5 cells/ml and cultured until ~80% confluency. A small linear scratch was created in the confluent monolayer by gently scraping with a sterile cell scrapper based on a described method[14]. Cells were thoroughly rinsed with 1 × PBS to remove cellular debris

and treated with different concentrations of PLGF. Cell proliferation was monitored at different time intervals – 30 min, 1–7 h and 24 h and images of the migrated cells were taken at all intervals using a digital camera (Nikon, Japan) connected to an inverted phase contrast microscope (IPCM; Radical Instruments, India). The extent of wound healing was determined by the distance traversed by cells into the denuded area.

Chorioallantonic membrane (CAM) assay

Fertilized chicken eggs on the second day of incubation were collected and incubated at 37°C with constant humidity. On embryonic day-three, a small hole was drilled at the narrow end to remove albumin. On the seventh day of incubation, a small square shaped window was made in the shell and NP encapsulated with and without growth factor were implanted on the membrane top. The window was further sealed and re-incubated up to appropriate time and angiogenesis was monitored[15] by analysing the vascular network through Image J software.

Skin irritation study – animal intracutaneous (intradermal) reactivity test

The study was conducted as per clause 6.4 of ISO 10993 Part 10 for Irritation and Skin Sensitization. Skin reaction at the site of application was subjectively assessed and scored once daily at 1, 24 and 72 h according to the following numerical system: 0 (no erythema), 1 (very slight erythema), 2 (well-defined erythema), 3 (moderate to severe erythema) and 4 (severe erythema).

In vivo wound healing

Wistar albino rats (*Rattus novergicus*, male), weighing between 190 and 230 g with the age range of 8–10 months were used for wound healing study. The animals were housed in standard environmental conditions: temperature (31 ± 1°C), humidity (60 ± 0.2%) and a 12 : 12 h light and dark cycle. The rats were used 7 days after arrival in the animal facility to allow them to acclimatize to laboratory environment. The experiment (including skin irritation) was carried out in central animal house facility, KMCH College of Pharmacy, Coimbatore (India) with the approval of Institutional Animal Ethical Committee for the experimental protocol and animal care. All care was taken to minimize the suffering of the animals.

Excision wound model: Excision wound was created on the animals using the method described by Morton and Malone[16]. The rats were anaesthetized with diethyl ether and placed on the operation table in their natural position. A wound of about 1.5 cm (width) × 0.2 cm (depth) was made on sterilized dorsal thoracic region of rats and separated into 9 groups containing 12 animals each. Animals were treated with different concentrations of GF loaded NPs. A total of 5 doses were given on alternative days up to 10 days. The wound contraction was studied by tracing the raw wound area on days 3, 6, 9, 12, 15, 18 and 21. Scar residue, time of complete epithelialization, area of contraction and percentage of wound closure were recorded.

The groups were as follows: group 1: control; group 2: positive control (treated with Becaplermin ointment, 2 mg); group 3: PLGA : poloxamer NPs (10 mg/ml); groups 4 to 9: Animals treated with 0.3, 0.4, 0.5, 0.6, 1.0 and 2 μg/ml PLGF.

The newly formed tissue as a result of complete wound healing was evaluated for its maximal breaking strength (MBS) by measuring its tensile strength (TS) using the following formula with tensiometer.

Tensile strength (N/cm^2) = breaking force (N)/area (cm^2); where area (cm^2) = thickness (cm) × width (cm) (ref. 17).

Histopathological studies: On the 7th and 21st day of treatment, three animals from each group were sacrificed by ether inhalation and skins of the wounds were removed. Specimens were preserved in a 10% fresh, neutral buffered formaldehyde for at least 24 h. Sections were prepared from each treatment group and stained with hematoxylin and eosin dyes and examined using a light microscope with 40 × objective lens.

Statistical analysis

Data are presented as mean ± standard deviation. Differences among experimental groups were analysed by applying one-way ANOVA.

Results and discussion

Characterization of PDGF-BB encapsulated PLGA : poloxamer blend NPs

Based on a previous study[11], PDGF-BB, a proangiogenic GF was loaded in PLGA : poloxamer blend NPs in the presence of Hp : BSA and various physico-chemical properties of NPs such as yield, encapsulation efficiency, size and morphology were studied.

The yield of NPs loaded with different concentrations of GF is given in Table 1. Due to the difference in quantity of aqueous layer used for incorporation of GF, the yield of NPs also varied. Literature suggests using 100 μl of aqueous layer for incorporation of 2 μg GF. However, an aqueous layer proportional to the concentrations of GF was used in this study. Hence, the availability of emulsion to forming NPs was low which in turn resulted in

less yield for the concentrations ranging from 0.3 to 0.6 μg. However, GF encapsulation efficiency was above 80% at all concentrations irrespective of the differences in yield, confirming its optimal loading.

Further, the NPs were characterized for their size and morphology using particle size analyser and FeSEM respectively. The mean diameter of particles was below 200 nm with the polydispersity index of <0.20, a unimodal particle population and a negative zeta potential. Varied zeta potential values were observed between particles encapsulated with GF in the presence (–34.4 mV) and absence (–26 mV) of stabilizers (Table 2). The higher surface negative charge of NPs loaded with Hp : BSA stabilized GF was due to the presence of Hp on the surface of NPs[13]. In line with the results of particle size analyser, FeSEM results also showed the homogenous particles with the sizes ranging between 100 and 200 nm. Morphology of the particles was regular and spherical in shape lining with the particles usually obtained by this method (Figure 1).

Biological evaluation of PDGF-BB loaded PLGA : poloxamer blend NPs – in vitro cytotoxicity and skin irritation studies

PLGA-based delivery system on the ability of PDGF-BB to promote cell proliferation and irritation potential in terms of erythema and edema of PLGA was studied through cytotoxicity and irritation as per ISO 10993 – parts 5 and 10.

As shown in Figure 2, the percentage of increase in cell population was measured after every 2, 4, 6, 8, 10, 12 and 24 h of treatment. The cell viability was close to 100% from 2 h of treatment for the range of GF : NPs. In some cases, the readouts from the GF treated groups were even higher than the control, suggesting the non-cytotoxic nature of PLGA : poloxamer and cell proliferation ability of GF. Among the different time periods and GF concentration, increased cell proliferation was observed from 2 h and continued up to 6 h. Further, the cell proliferation was maintained between 100% and 120% till 24 h treatment (Figure 2) for all the different concentrations of GF.

Table 1. Percentage yield and encapsulation efficiency of PDGF-BB loaded PLGA : poloxamer blend NPs

Concentration of PDGF-BB loaded in PLGA : poloxamer (μg/ml)	Yield (%)	Encapsulation efficiency (%)
0.3	66.0	83
0.4	64.7	81
0.5	65.3	88
0.6	64.3	85
1.0	74.3	86
2.0	95.3	88

The difference in cell proliferation at different time intervals suggests the release of surface bound GF at different time points. In addition to GF-treated cells, PLGA : polaxamer and polaxamer treated cells also stimulated proliferation. This could be due to the ability of poloxamer in stimulating mitochondrial respiration of the proliferating cells[18,19]. Hence, it cannot be correlated with the viability of GF-treated groups. On the whole, proliferation of cells was found to be dependent on the GF concentration present on the surface of nanoparticles. The result of skin irritation study confirms the non-irritant nature of PLGA and PLGF particles as the primary irritancy index determined was 0 in all the cases. Moreover, none of the assayed formulations showed signs of erythema, edema or swelling on the intact rabbit skin at the end of the study (Table 3).

PDGF-BB release profile from PLGA : poloxamer blend NPs

The kinetics of release of PDGF-BB from PLGA : poloxamer blend NPs is shown in Figure 3. PDGF-BB at all different concentrations exhibited initial burst release between 33% and 42% and there were no significant release up to five days since the GF was released very slowly. Subsequent to that, there was a sustained GF release between 27 and 38 days. Depending on the concentration of GF encapsulated in the NPs, the release profile decreased exponentially. The results of the study agreed with the previously reported release kinetics[11,13], including high initial burst release. In addition, the release pattern also exhibited resemblance to the characteristics of PLGA-mediated drug release, viz. (i) an initial drug release associated with diffusion of encapsulated molecule localized near the particle surface, (ii) a lag period where the drug is released very slowly and (iii) a controlled release phase associated with polymer matrix degradation[20,21]. The lag phase corresponds to the time of hydration of polymer matrix and generation of water channel, representing the beginning of polymer erosion phase. However, the presence of poloxamer on the surface of NPs alters the hydration property and facilitating water infiltration thereby promotes the formation of channels[22]. As a result, PLGA : poloxamer particles exhibit faster onset of second and third phases of drug release. In the present study, relatively high ratio between BSA and GF (70 : 1) could have enhanced the GF release from the particles.

As release of GF can occur by either passive (dissociation and subsequent diffusion from the surface of NPs) or by active (cell-mediated) means, we next studied the behaviour of NPs in releasing GF in the presence of cells. As shown in Figure 4, the recovery of GF at 2 h time was between ~3.5% and ~9%. Upon increasing the incubation time, the release pattern varied from low, moderate

Table 2. Size, polydispersity index (PI) and zeta potential of PDGF-BB loaded PLGA : poloxamer blend NPs

NPs	Size (nm)	PI	Zeta potential (mV)
PLGA : poloxamer	120.1 ± 0.4	0.192–0.215	–26.0 ± 1.2
Hp, BSA, PDGF-BB loaded PLGA : poloxamer	177.6 ± 7.6	0.269–0.377	–34.4 ± 1.0

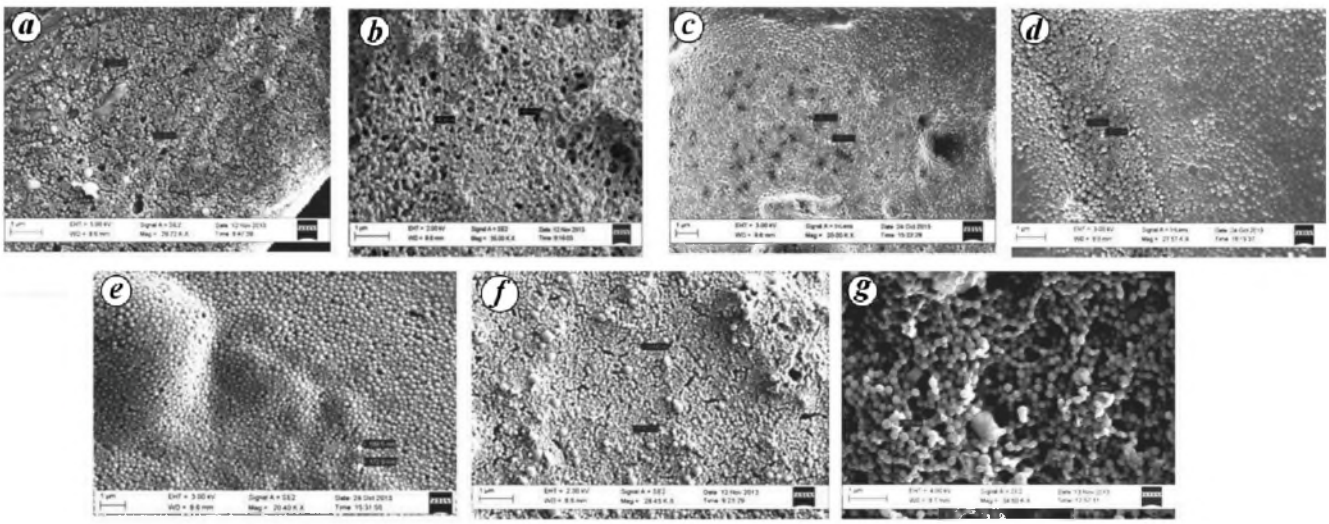

Figure 1. FeSEM images of PLGA : poloxamer blend nanoparticles (NPs) loaded with PDGF-BB (colloidal suspension). ***a***, PLGA : poloxamer blend NPs; ***b***, PLGF 0.3 μg/ml; ***c***, PLGF 0.4 μg/ml; ***d***, PLGF 0.5 μg/ml; ***e***, PLGF 0.6 μg/ml; ***f***, PLGF 1.0 μg/ml and ***g***, PLGF 2.0 μg/ml.

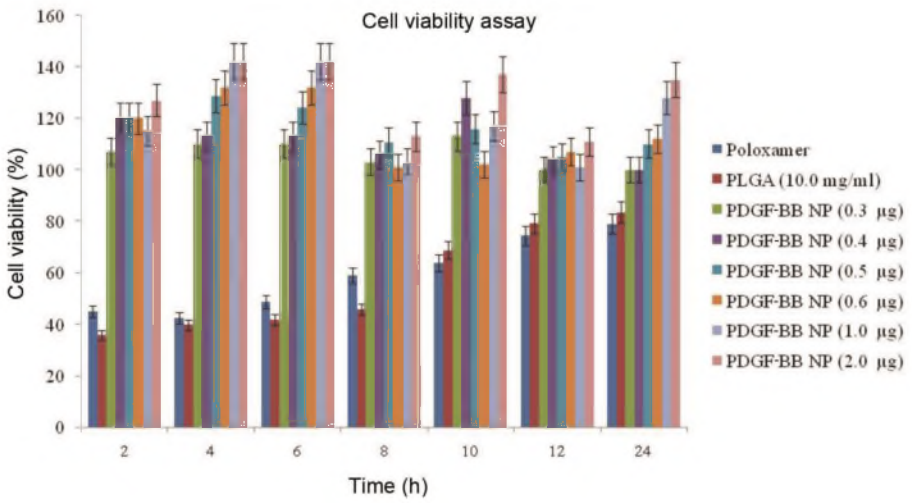

Figure 2. Cell viability measured by a metabolic activity assay (MTT) in L929 fibroblast cell in the presence of increasing concentrations of PDGF-BB loaded PLGA : poloxamer blend NPs and PLGA NPs. The nanoparticle suspensions in cell medium were incubated with the cells between 2 and 24 h at 37°C. Bars represent means ± SD, $n = 3$.

and burst, based on the GF concentration on the surface of NPs. For lower concentrations, the release was between 3.5% at 2 h and 6% at 48 h. In contrast, multifold release was observed for NPs loaded with high concentrations of GF, i.e. 1 and 2 μg/ml (Figure 4). Maximum recovery of ~40% was observed after 48 h incubation with cells. Further, cellular protein was assessed to evaluate GFs that were internalized during incubation. In contrast to extra cellular medium, there is no detectable level of GF measured within the cells which shows that either the duration of contact is not sufficient for internalization of NPs or it would have degraded by the cells and/or masked from antibodies used in ELISA.

The controlled release abilities of the blend NPs were confirmed from the present study; hence it was further evaluated for wound healing applications.

In vitro wound healing assay

The objective of the present study was to investigate the ability of GF encapsulated blend NPs for angiogenesis and wound healing activity. Hence an *in vitro* scratch wound model was created in L929 mouse fibroblast monolayer and the response of the wounded cells to the GF in cell-free surface was analysed using inverted phase contrast microscope (IPCM).

The cells were treated with PLGA : poloxamer NPs encapsulated with (0.3, 0.6, 1 and 2 μg/ml) and without GF. Cells were allowed to migrate into the denuded area for 12–48 h at 37°C. GF treated cells irrespective of the difference in concentrations, migrated more quickly into the denuded area within 12 h and the wound closed before 24 h when compared to control cells (Figure 5). To identify the concentration required for cell migration and healing, the study was conducted at 0 to 12 h. As shown in Figure 6, early migration was noticed in cells treated with 0.4 μg/ml of GF at 6 h and the wound healed completely by 10 h of treatment. The wound treated with 0.6 μg/ml of GF however healed the wound by 8 h. These results were in line with the cell proliferation (Figure 2) assay where higher cell population was observed at 4 h of treatment for all different concentrations of GF suggesting the healing of wound by both migration and proliferation of cells. Also, 0.4 μg/ml is identified to be the minimum concentration required for migration and 0.6 μg/ml for healing the wound.

Angiogenesis stimulating capability was investigated using 2-day-old fertilized chicken eggs. After reaching CAM on seventh day, 10 μl of GF encapsulated NPs (0.3–2 μg) was placed and observed for vascular network change on every alternative day through a small square window. On the tenth day of treatment, drastic change was observed in the length and width of blood vessels where the width of blood vessel of the treated eggs was higher than that of control eggs (Table 4). This could be due to the surface bound GF. However, because of the inability of CAM to degrade NPs, a key requirement for the sustained release of GF, there is no significant difference in capillaries of treated and control eggs. Therefore, *in vivo* studies using rat excision wound model were conducted to study the wound healing/angiogenesis stimulating ability of drug delivery device fabricated in the present study.

Table 3. Primary skin irritation study

Treatment groups	Erythema/edema scores after the following hours of treatment			
	1	24	48	72
Control 01	0/0	0/0	0/0	0/0
PLGA	0/0	0/0	0/0	0/0
PLGF 0.3 μg	0/0	0/0	0/0	0/0
PLGF 0.6 μg	0/0	0/0	0/0	0/0
PLGF 1.0 μg	0/0	0/0	0/0	0/0
PLGF 2.0 μg	0/0	0/0	0/0	0/0
Total	0/0	0/0	0/0	0/0

The primary irritation index of samples (PLGA and PLGF) was calculated to be 0.00; No signs of erythema/edema were observed on the skin of rabbits ($N = 3$).

Table 4. Morphological changes in the vascular network of CAM treated with PLGF NPs

Groups	Blood vessel (mm)		No. of capillaries
	Length	Breadth	
Control	88.6	2.9	5.0
0.3	143.6	6.7	4.5
0.4	222.0	9.5	6.0
0.5	185.3	10.4	10.0
0.6	196.2	6.5	3.5
1	220.6	12.1	6.0
2	178.8	8.2	6.0

Values are average of 2.

Table 5. Tensile strength (TS) of excision wounds treated with GF encapsulated PLGA : poloxamer NPs

Treatment groups	TS (N/cm^2)
Untreated	8.84 ± 0.5
PLGA : poloxamer	8.13 ± 0.31
Becaplermin treated	15.6 ± 0.32
0.3 μg	11.3 ± 1.28
0.4 μg	11.2 ± 1.19
0.5 μg	11.6 ± 0.96
0.6 μg	13.9 ± 1.6
1.0 μg	15.8 ± 1.03
2.0 μg	15.4 ± 0.67

Values are presented as mean ± SD, $n = 3$.

Excision wound model

To evaluate the *in vivo* performance of the GF releasing NPs, excision skin wounds were created on the back of

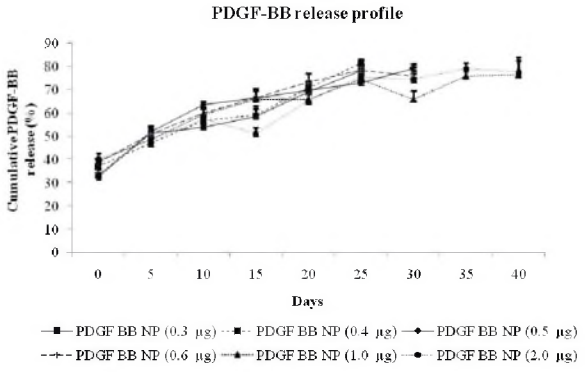

Figure 3. Release profile of GF from PLGA : poloxamer blend NPs. Data are represented as mean ± SD, $n = 3$.

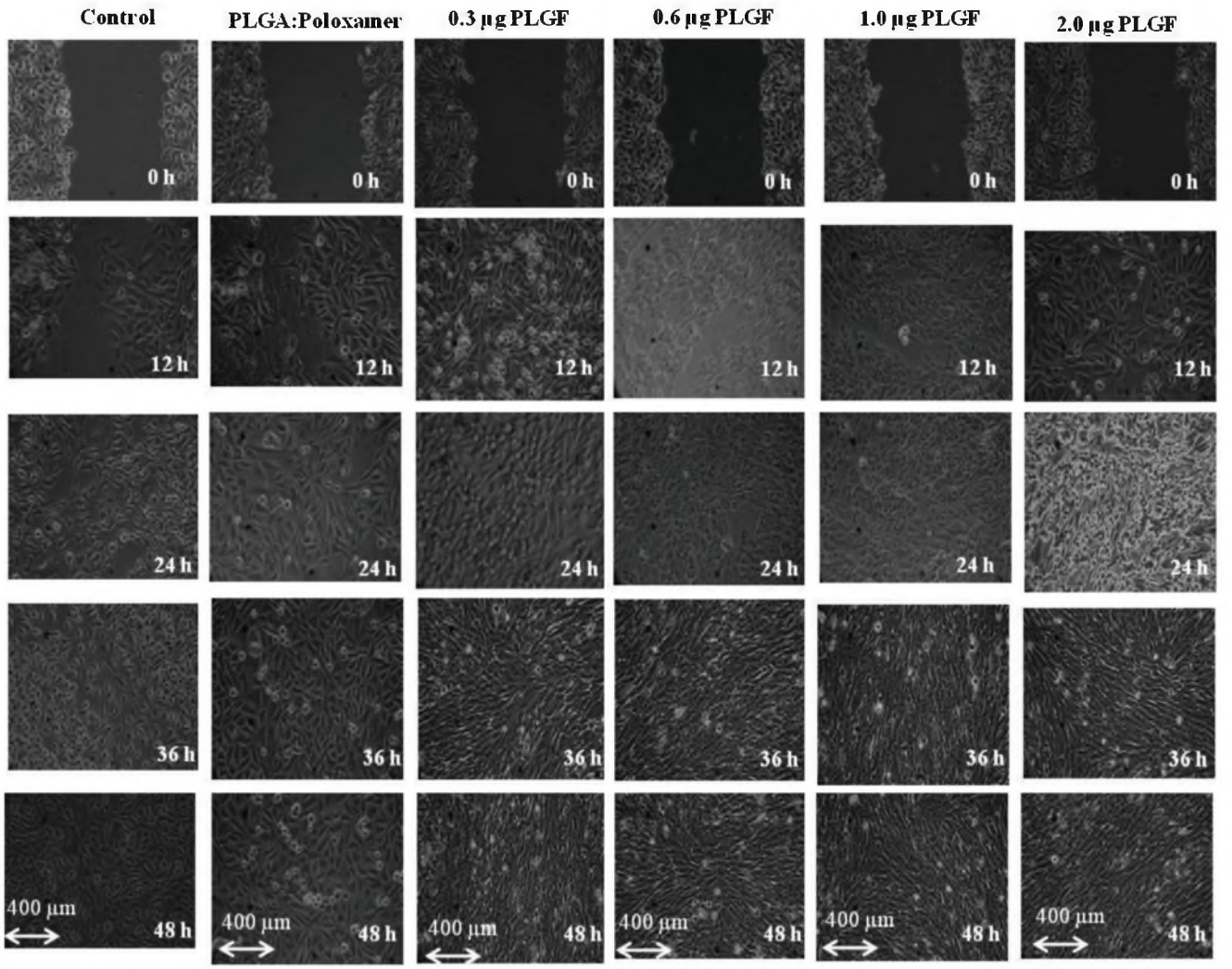

Figure 4. Phase contrast microscopic images of scratch-wounded fibroblast cultures show the increased migration of fibroblasts cells when treated with different concentrations of GF loaded NPs. Magnification: 40×, scale bar: 400 µm.

rats. The gross appearance of each wound was observed after 1, 2 and 4 weeks of treatment. As shown in Figure 7, the wounds of control animals contracted from 19.5% to 66.4% from day 3 to 12 and 78.1% to 94.7% from day 15 to 21. Complete epithelialization and healing were seen on day 26. In contrast, becaplermin and GF-treated rats had almost a similar pattern of wound contraction and epithelialization and complete healing was observed between days 12 and 16. The mean epithelialization period and scar area observed with becaplermin treated rats were 10.3 and 62.5 mm^2, while that of GF treated animals were between 12.3 and 9.7 and 77.9 and 64.1 mm^2 respectively, for various concentrations of GF, thus suggesting that the healing pattern was on par with commercial ointment. To differentiate the healing pattern between different treatment groups, tensile strength (TS) of newly formed tissues was analysed (Figure 8). Tissues exhibited different TS of 11.2–11.6 N/cm^2 and 13.9–15.8% N/cm^2 for low (0.3–0.5 µg/ml) and high concentration GF (0.6–2 µg/ml) respectively (Table 5). The differences in the healing rate were found to be significant between the concentrations that is between low and high but not within the concentrations. Moreover, the TS of high concentration GF-treated wounds were comparable with becaplermin.

Histopathological evaluation

For histopathological studies, granulation tissues after 7 and 21 days of treatment were excised and investigated for inflammatory cells, neovascularization and collagen deposition. On day 7, the regenerating granulation tissues of GF encapsulated NPs and becaplermin-treated animals exhibited prominent vascularity and accelerated granulation tissue formation relative to control groups. On day 21, the tissues of the treated animals showed abundant collagen deposition, neovascularization with few inflammatory cells and normal epidermis (Figure 7). Dense and thick fibrocollagenous stroma and congested vessels

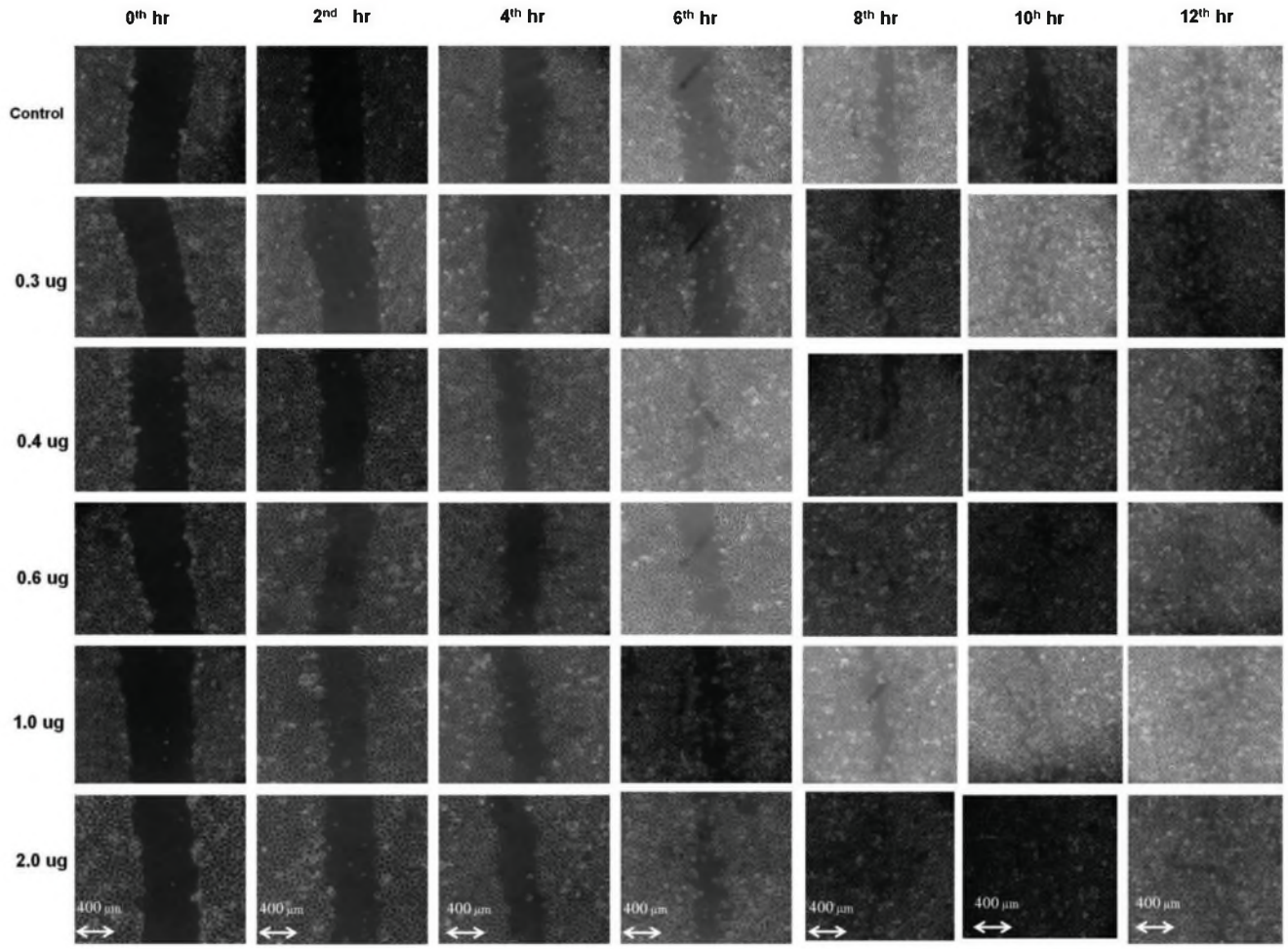

Figure 5. Wound scratch assay at early time points: phase contrast microscopic images of scratch-wounded fibroblast cultures show the early migration of cells at 0.4 µg/ml concentration at 6 h when compared to control cells. Magnification: 40×, scale bar: 400 µm.

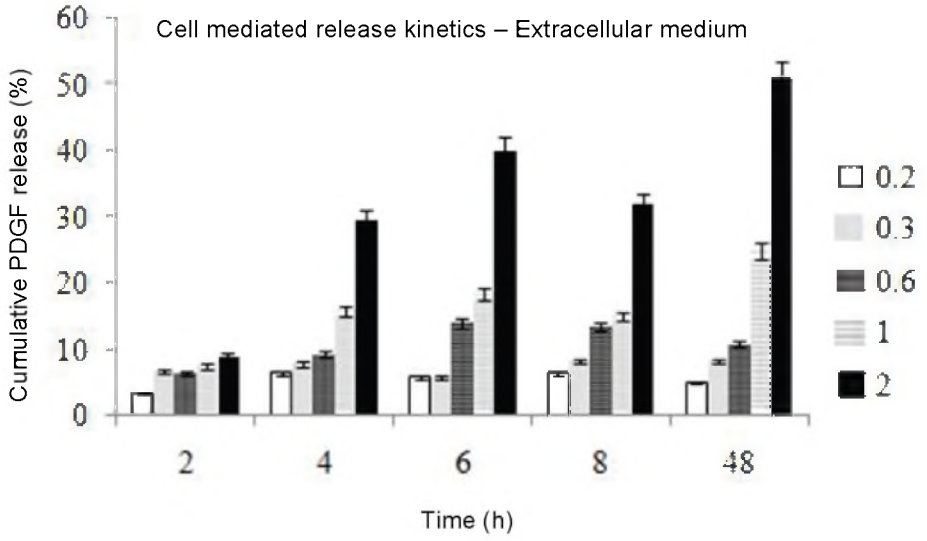

Figure 6. Release profile of PDGF-BB in the presence of L929 mouse fibroblast cells. Data are represented as mean ± SD, $n = 3$.

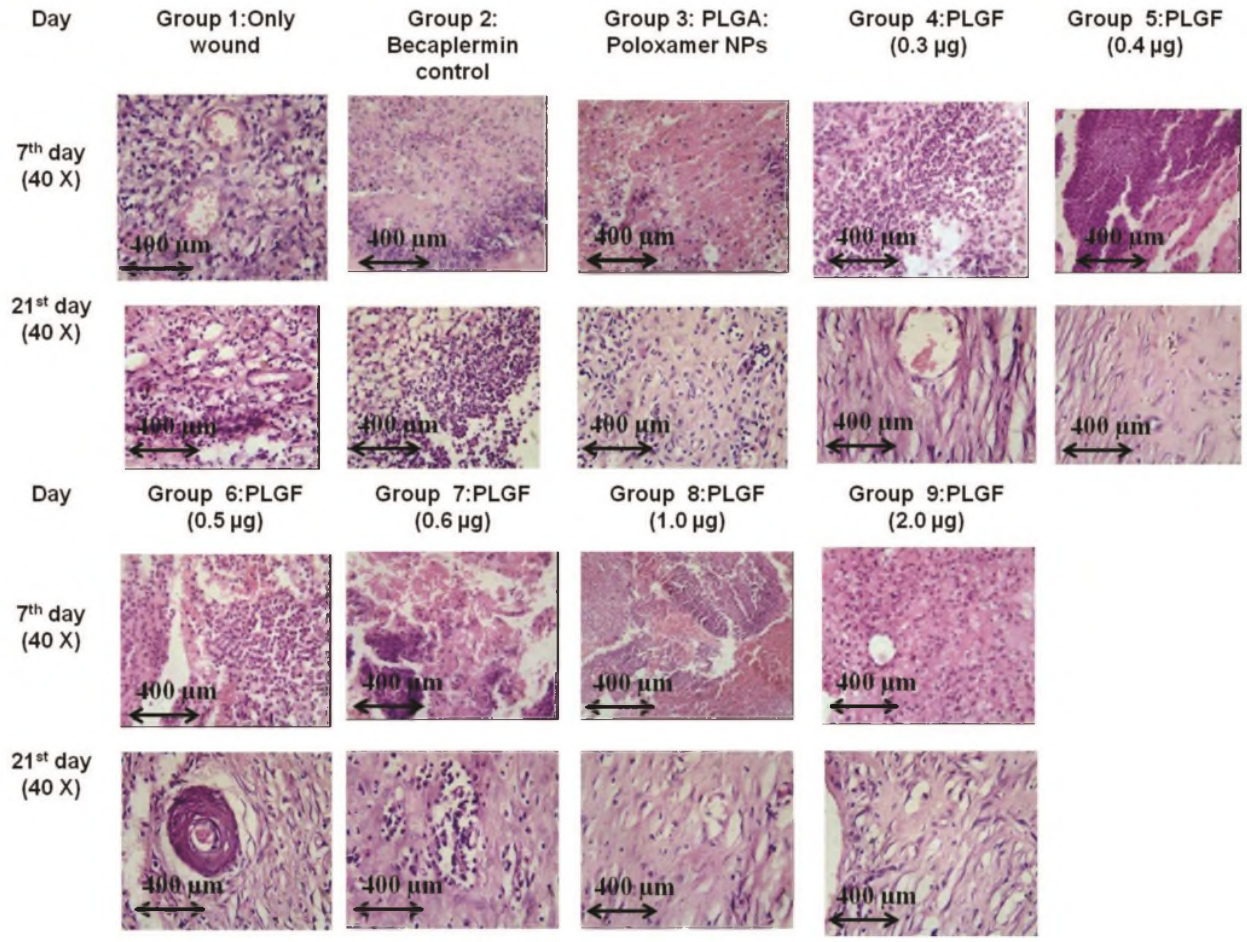

Figure 7. Histopathological analysis of the animals treated with PDGF-BB loaded NPs. Scale bar: 400 µm.

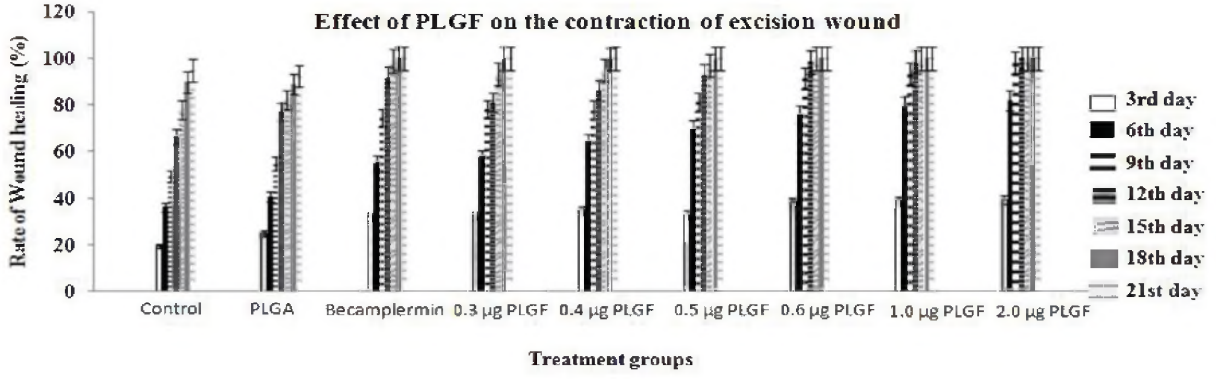

Figure 8. Effect of PLGF on the contraction of excision wound created in rat model. Values are mean ± SD of 3 rats in each group. Statistical analysis was carried by one way ANOVA followed by Dunnett's test for multiple comparison.

formation were also observed in the treated groups which were negligible in control groups.

Collagen, a biomechanical component of connective tissue is responsible for tissue strength. Hence, the high TS seen in GF and becaplermin-treated rats could be because of the high collagen content in wound lesion. Moreover, granulation tissues of becaplermin and GF (1 and 2 µg/ml) appeared thick when compared to the control and PLGA treated rats. Complete closure of epithelium and thickened granulation tissue suggest a transition from inflammation to proliferation phases of wound healing. This in turn matches with healing usually stimulated by PDGF-BB such as fibroblast proliferation, collagen production and neovessel formation[23].

Summary

In line with earlier studies, controlled PDGF-BB release from PLGA : poloxamer blend NPs prepared through modified solvent method was achieved in this study. The NPs promoted fibroblast proliferation under *in vitro* conditions and healed wound before 24 h. Under *in vivo* conditions, an accelerated wound healing was achieved before 12–16 days on an excision rat skin wound from the concentration of 0.6 μg/ml by improving epithelium regeneration, collagen deposition and functional tissue remodelling. From the present study, it may be summarized that if the controlled delivery and improved wound healing are proved through clinical trials, they will have promising potential for treatment of chronic wounds in future.

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