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SYNTHESIS AND CHARACTERIZATION OF BIOACTIVE PLGA NANOPARTICLES - WOUND HEALING IN MICE MODEL

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Abstract

Wound therapy remains a significant global health challenge. In this study, we investigated the use of poly (lactic-co-glycolic acid) (PLGA) nanoparticles for wound healing. PLGA, known for its biodegradability and biocompatibility, was employed to deliver large biomolecules, including proteins and vaccines. Our method involved dissolving 50 mg of PLGA in 4 mL of dichloromethane (DCM) to create an organic phase. The resulting nanoparticles were lyophilized and applied to wounds in a mouse model. Histological analysis revealed accelerated wound healing on Days 3, 6, and 8. Notably, PLGA nanoparticles demonstrated promise as a controlled, long-acting drug delivery system. In our study, we explored the potential of poly (lactic-co-glycolic acid) (PLGA) nanoparticles for wound healing. By acting as carriers for therapeutic agents, they facilitated sustained drug availability at the wound site. This gradual release contributed to neovascularization and cellular proliferation, crucial for tissue repair. Beyond wound healing, PLGA nanoparticles hold promise in various biomedical applications, including tissue engineering and regenerative medicine. However, clinical trials are essential to validate their efficacy in human patients.

Keywords: Biocompatible, Ketamine, Medication, Nanoparticles, Neovascularization.

1. INTRODUCTION

Normal anatomical structure and function are disrupted in a wound and wound healing is a dynamic, intricate process that involves restoring anatomic continuity and function.(Appoo, Christensen and Somayaji, 2024) Based on factors like appearance, number of disrupted layers, cause of injury, and healing process, wounds have been categorized.(Govindarasu, Prathap and Govindasamy, 2022) Furthermore, wounds can be classified as acute (full healing and little scarring) or chronic (slow healing and recurring tissue injury) depending on the repair process.(Yilmaz Akyaz, Cevizci and Sengul, 2024) A complex biological process, wound healing entails a series of overlapping and interconnected physiological activities.(Bakadia *et al.*, 2024)

Wound healing is extremely difficult in the absence of active therapy, especially in chronic wounds with inadequate blood flow and localized edema.(Wang *et al.*, 2024) In an effort to speed up wound healing and increase the range of wound types for which they can be used, numerous devices, dressings, medications, and delivery methods have been thoroughly studied.(Govindarasu, Prathap and Govindasamy, 2022; Elumalai, Ezhilarasan and Raghunandhakumar, 2023) The urgency of research and development in the field of wound care is highlighted by both scientific and industrial realities.(Han *et al.*, 2024) Numerous powerful wound-healing agents have been found, but the majority of them are delicate and/or sensitive to in vivo environments, meaning that effective administration of these agents is necessary to enhance the current wound-care regimens.(El-Saied *et al.*, 2024) In fact, this lowers the cost, boosts efficacy, and is applicable to a wider spectrum of patients and

wounds.(Govindarasu, Prathap and Govindasamy, 2022; Elumalai, Ezhilarasan and Raghunandhakumar, 2023; Raghunandhakumar, Ezhilarasan and Shree Harini, 2024) For these uses, one of the best drug delivery methods known is poly(lactic-co-glycolic acid) (PLGA). It has been effectively demonstrated that PLGA nanoparticles (NP) are effective medication and biomolecule carriers for the treatment of a variety of illnesses.(Jahn *et al.*, 2024) One method for encasing hydrophobic or poorly soluble medications is PLGA NP. PLGA NP might offer several chances to investigate novel approaches for the regulated and prolonged local release of loaded medications for the purpose of wound healing.(Singh *et al.*, 2023)

A popular biodegradable polymer in medical applications is PLGA, a copolymer of polylactic acid and polyglycolic acid. Because of its great biocompatibility, PLGA has been given parenteral administration approval by the FDA.(Yang *et al.*, 2023) The capacity to modify surface and physio-chemical properties, the commercial availability of GMP PLGA, its favorable degradation and its sustained drug release made PLGA the most intriguing polymeric drug carrier for a variety of therapeutic applications.(Hernández-Giottonini *et al.*, 2023) When PLGA is hydrolyzed, its ester connections are broken, forming monomers of lactic acid and glycolic acid that are readily metabolized by the Krebs cycle.(Kotelnikova, Shipunova and Deyev, 2023) Lactate is crucial to the processes involved in wound healing.(Govindarasu, Prathap and Govindasamy, 2022; Elumalai, Ezhilarasan and Raghunandhakumar, 2023; Kumar, Ezhilarasan and Veeraiyan, 2024; Raghunandhakumar, Ezhilarasan and Shree Harini, 2024) Hypoxia is a physiological complication of the wound healing process that results from damage to the microcirculation and from the inflammatory cells' increased oxygen consumption.(Zhu *et al.*, 2023) A significant amount of lactate is produced as one of the byproducts of the anaerobic respiration including glycolysis, which is encouraged by the hypoxic environment.(Gimondi *et al.*, 2023)

In an effort to destroy bacteria and other invasive microorganisms, the quickly proliferating newly recruited and preexisting cells, activated leukocytes, and macrophages also release lactate as a result of an oxidative burst that produces reactive oxygen species.(Gimondi *et al.*, 2023; Rodponthukwaji *et al.*, 2023) Exogenous lactate administration has the potential to expedite the processes of angiogenesis and wound healing; PLGA appears to be the most appropriate polymer for supplying lactate in order to expedite reparative angiogenesis and wound healing.(Govindarasu, Prathap and Govindasamy, 2022; Elumalai, Ezhilarasan and Raghunandhakumar, 2023; Kumar, Ezhilarasan and Veeraiyan, 2024; Raghunandhakumar, Ezhilarasan and Shree Harini, 2024; Shree Harini, Ezhilarasan and Mani, 2024) Because lactic acid is hydrophobic, PLA polymers high in lactides are less hydrophilic, absorb less water, and break down more slowly.(Pinto *et al.*, 2024) None of the previous studies done on PLGA examined its therapeutic activity in the healing of wounds. Therefore, the aim of our current study is to address the therapeutic effects of PLGA on the process of wound healing in mice.

2. MATERIALS AND METHODS

Synthesis of Kaempferol + PLGA nanoparticles

To create an organic phase, dissolve kaempferol in 4 mL of DCM and 50 mg of PLGA. At 250 rpm, this organic phase was agitated for 15 minutes. After adding 20 mL of a 0.5% PVA solution dropwise and ultrasonography (220 V) in an ice bath for five

minutes, the organic phase was completed. After being lyophilized to produce a dry powder, the produced nanoparticles were sent for analysis. FTIR, X-ray diffraction and in Vivo wound healing potential were used to characterize the drug-loaded PLGA NPs.

Fourier transform infrared spectroscopy (FTIR) study

Any substance or medication's infrared spectrum can provide information about the groups that are present in that specific compound. The IR spectra of the nano formulation, PLGA, and kaempferol were acquired. After freeze-drying the nanoparticle formulations, the powder samples were combined with KBr to produce pellets. FT-IR spectrometers (Perkin Elmer) were used to record FT-IR spectra in the absorbance mode. Varied groups were interpreted to be present based on the varied peaks in the infrared spectrum.

X- ray diffraction analysis

The pure drug and the nanoparticle formulation's crystallinity were determined by X-ray diffraction analysis, which was carried out using a Philips PW 3710 x-ray diffractometer (XRD) equipped with a nickel filter and copper target (Philips Electronic Inst, Holland). A flat surface was achieved by mounting powders on glass-bottomed aluminum stages. Every sample's XRD pattern was measured between 10 and 50 degrees. 2-theta with a dwell period of one second between steps and a step increment of 0.1 2-theta degrees

Scanning Electron Microscope (SEM)

By applying the nanoparticle dispersion to recently split mica and letting it dry, SEM studies were carried out. Using the sample, a thin layer of Au was sprayed onto these mica substrates. Without filtration or purification, samples were examined using scanning electron microscopy (SEM; JEOL 5800LV). ImageJ was used to measure the particle size. Based on the measurements of 100 randomly selected particles, the mean diameter for each sample was determined.

Animals and Wound Creation

All of the study's experiments were planned and carried out with consent from the ethics committee and in compliance with animal welfare regulations. Wound was inflicted on mice's skin before which each mouse received an appropriate injection of Ketamine/ Xylazine (70/10 mg/kg b.w.) anesthesia based on body weight throughout the development of the wound model. Following the anesthesia, depilatory cream was used to remove the hair from each mouse's back, and 75% alcohol was used to clean the area. Next, we created a 2 cm-diameter circular wound on the back of each mouse using sterile medical scissors, which allowed us to remove all of the skin, including the dermis and epidermis, down to the muscle layer. On the wound the Nanoparticles was applied topically daily and repeated for 7 days. At Day 8, animals were sacrificed and skin tissue collected were stored in 10% formaldehyde and sent to histopathological examination.

Summary of Methods

Kaemferol dissolved in 4 mL of DCM + 50 mg of PLGA to form an organic phase. This organic phase was stirred for 15 min at 250 rpm. In the organic phase, 20 mL of 0.5% PVA solution was added dropwise and ultrasonication (220 V) in an ice-bath for 5 minutes. The synthesized Nanoparticles were lyophilized to obtain a dry powder and sent for characterization.

3. RESULTS

Shows the results of an F.T.I.R. research conducted to verify the compatibility of the chosen polymer medication, kaempferol, and nanoparticle formulation. The I.R. studies yielded spectra ranging from 3600 cm-1 to 600 cm-1. The kaempferol drug's significant peaks attest to the existence of distinct groups.

The peak occurs at 3695.96 cm-1 when O-H (stretching) aromatics is present; 2889.56 cm-1 when C-H (stretching); 1581.51 cm-1 when ketone stretching. (alkanes stretching) was at 1405.04 cm-1 and alkyl amine stretching at 1074.60 cm-1 and C=O=C bending at 631.78 cm-1.

It was established that there was no significant shifting or loss of functional peaks across the spectra of the drug, polymer, and drug-loaded NPs. Instead, the peak at 3695.96 cm-1 grew larger and flatter, suggesting that the hydrogen bond was strengthened.

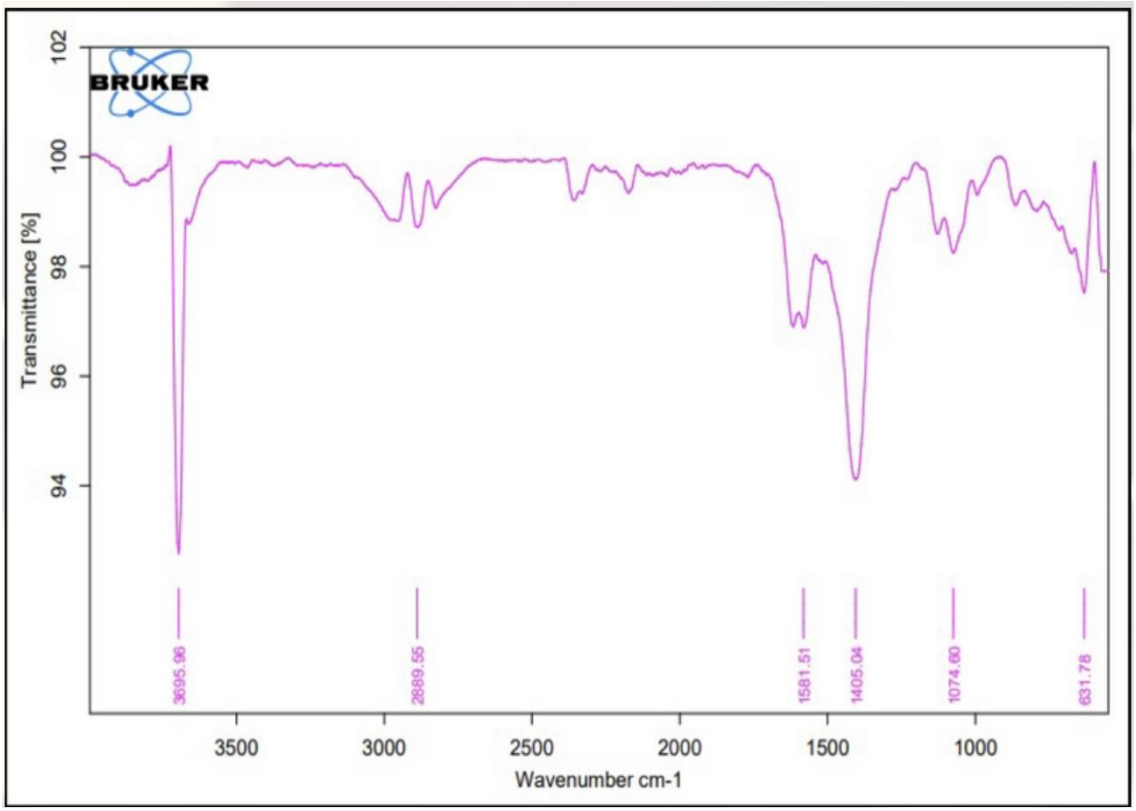


Figure 1: XRD pattern of PLGA nanoparticle with kaempferol

PLGA nanoparticles filled with kaempferol. The XRD pattern of kaempferol showed distinct, strong peaks at around 25θ and 50θ. The high-intensity peaks in PLGA (50:50) formed a dome-shaped region and ranged from 30 to 35. The XRD pattern of drug-loaded nanoparticles display the strong and pointed drug peaks.

It actually looks a lot like the PLGA pattern with the high intensity peaks. It was determined that the acquired sample was largely amorphous rather than crystalline due to the extremely limited area under the peaks.

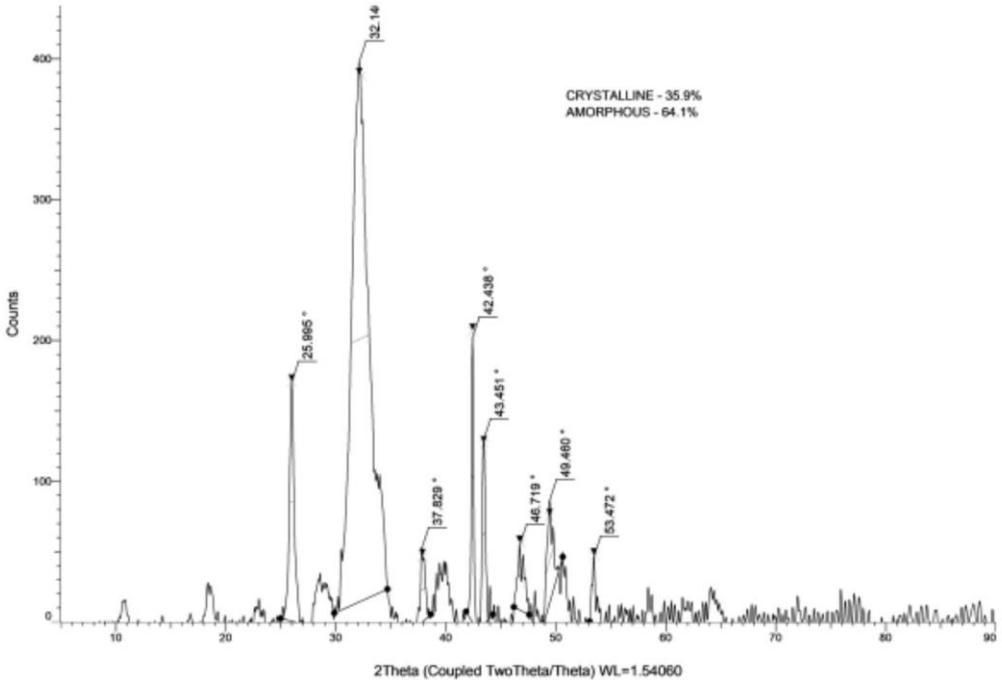
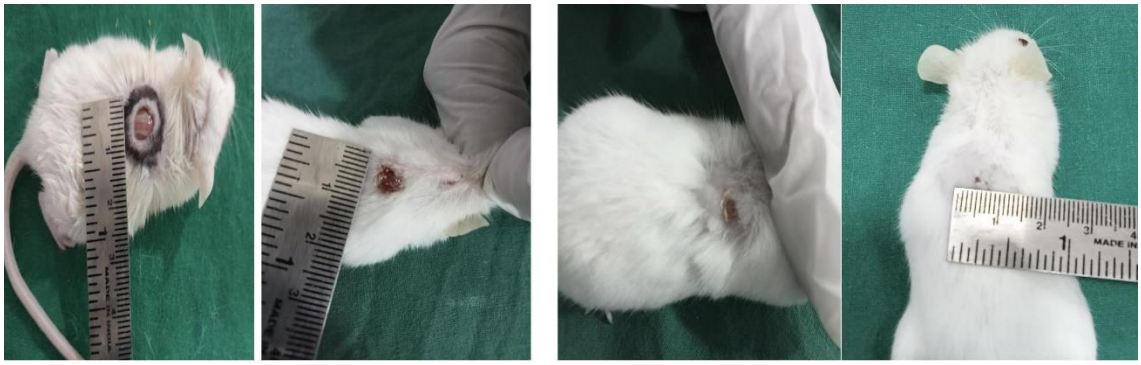


Figure 2: FTIR Pattern of PLGA nanoparticle with kaempferol

Prepared wound healing formulation produced a size within the nano range, and all of the formulations' size distributions were essentially monodisperse with PDIs ranging from 0.16 to 0.36. Different formulations with respect to drug/polymer ratios, external phase volume, and agitation speed. Using a 25 ml external phase volume and an agitation speed of 20,000 rpm, the kaempferol-PLGA nanoparticle size ranged from 330 to 470 nm. The resulting nanoparticles were found to have a spherical form and a rather smooth surface according to SEM studies.

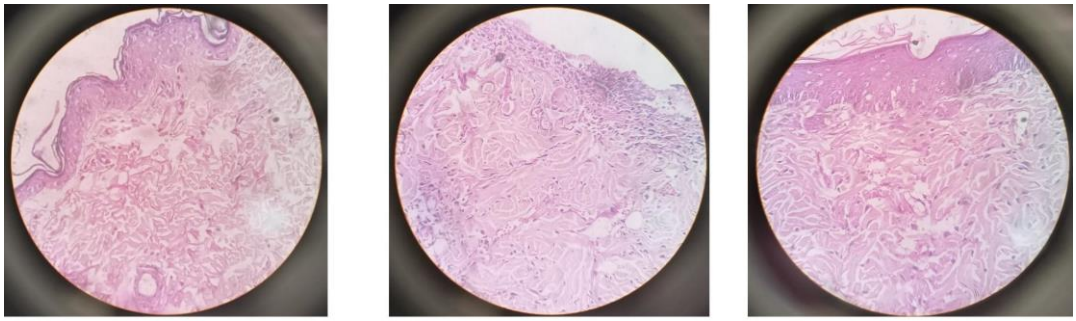
Figure 3: SEM image of PLGA nanoparticle with kaempferol

Wound healing activity was characterized using visual and measurement analysis of the healing and repair of the epithelial tissue surface of mice. Healing of wounds was observed on Day 3, 6 and 8. Size of wound was observed using a metal ruler and healing of wound was determined. As days progressed healing of wounds occurred rapidly.



Day 1 | Day 3 | Day 6 | Day 8

Figure 4: Healing of wound in mice model



Control mice | Treated mice | Nanoparticles Treated mice

Figure 5: Histo pathological images of wound in mice model

4. DISCUSSION

Numerous investigations have discovered proof of the use of PLGA NP for the regulated release of antibiotics in the context of wound healing.(Yuan *et al.*, 2024) S. aureus can cause a variety of illnesses, from simple skin infections to skin infections following surgery.(Ezhilarasan *et al.*, 2024) PLGA NPs are a promising tool in wound healing and have prominent antibacterial action against S. aureus and other harmful bacteria.(Arvas *et al.*, 2024)

Previous studies used curcumin with PLGA nanoparticles

In Chinese and Indian Ayurvedic medicine, curcumin (CC) is a well-known medication for mending wounds.(Prathap *et al.*, 2021) When used topically, CC has been shown to be a promising antibacterial and wound-healing agent in both normal and diabetic-impaired wounds.(Agrawal *et al.*, 2023) CC with PLGA produced a lesser rate of wound healing than kaempferol with PLGA utilized in our study.(Han *et al.*, 2023) On the 8th day mice utilized in our study had a good healing and circumference of wound was 1 mm whereas on the same day CC with PLGA treated mice had a wound circumference of 5 mm. Besides the fact that on the 1st day the circumference of the wound was about 8 mm in both cases. Better wound healing was found with kaempferol with PLGA treated mice. Apart from visual and measurement aids, histo pathological examination of tissue of mice was also done to confirm the proper repair

of wound on treatment with kaempferol with PLGA nanoparticles. None of the previous studies done on wound healing with PLGA nanoparticles took histo pathological examination into consideration for their study. Our results are favorable in ensuring the proper healing of wound in mice as the epithelial layer of untreated mice was not in a uniform consistency. It had surface blebs. Kaempferol with PLGA treated mice had no surface blebs on tissue examination.

Limitation of our study is concerned with use of mice models because mice are readily available and simple to care for, even in tiny animal facilities, several researchers have employed them as animal wound models. It results in a significant drawback because rodents have loose skin and their wounds heal primarily by contraction, which makes the end results to be biased.

5. FUTURE SCOPE OF RESEARCH

Depending on the severity of the wound, current active wound care mostly consists of dressing, antibiotic, and stimulant for healing.(Varshan and Prathap, 2022) A therapy that satisfies all three of these essential requirements in a single dosage form would significantly reduce costs and hasten the healing of chronic wounds.(Arthanari, Sureshbabu, *et al.*, 2024) Creation of a single multifunctional PLGA dosage form with all the necessary requirements would be a tremendous accomplishment to have properties like antibacterial, protect hydration, allow gas exchange, and stimulate active healing.(Arthanari, Senthilkumar, *et al.*, 2024) These qualities may also lower costs and speed the healing of chronic wounds.

6. CONCLUSION

PLGA nanoparticles are a great substitute for creating a medication with a regulated, long-acting release for wound healing. These findings suggested that the biological wound healing activity in the mice model would be greatly enhanced by produced PLGA nanoparticles.

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Conflict Of Interest

The authors hereby declare that there is no conflict of interest in this study.

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