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Biocompatibility of Amoxicillin and Diclofenac Sodium Loaded Poly-lactic Acid Microspheres: A Descriptive Study

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ABSTRACT:

The safety of any drug delivery system destined for intra-oral application extends beyond its pharmacological efficacy to include its interaction with living tissues and blood components. While localized drug delivery systems (LDDS) such as poly-lactic acid (PLA) microspheres have shown great promise for sustained release of therapeutic agents in dental and maxillofacial applications, their translation into clinical use depends on thorough biocompatibility validation. In particular, for use in extraction sockets—an environment rich in blood supply and active tissue remodelling—understanding the cellular and haematological responses to these biomaterials is critical.

This study demonstrated that diclofenac sodium–loaded PLA microspheres exhibit favorable cytocompatibility in fibroblast cultures at concentrations up to 125 µg/mL, indicating their potential for safe intraoral application in soft tissue environments such as extraction sockets. However, a marked increase in hemolytic activity at higher concentrations underscores the importance of controlled dosing and formulation optimization to minimize blood-contact cytotoxicity. The results suggest that while the microspheres are biocompatible with oral fibroblasts, caution must be exercised to avoid systemic exposure or high local concentrations that could compromise erythrocyte integrity. Future improvements, such as surface modification or co-polymer blending, may enhance the safety profile and broaden the clinical applicability of this localized drug delivery system.

1. Introduction

The safety of any drug delivery system destined for intra-oral application extends beyond its pharmacological efficacy to include its interaction with living tissues and blood components. While localized drug delivery systems (LDDS) such as poly-lactic acid (PLA) microspheres have shown great promise for sustained release of therapeutic agents in dental and maxillofacial applications, their translation into clinical use depends on thorough biocompatibility validation [1,2]. In particular, for use in extraction sockets—an environment rich in blood supply and active tissue remodelling—understanding the cellular and haematological responses to these biomaterials is critical [3].

Amoxicillin and Diclofenac Sodium are widely used in oral surgery for infection control and inflammation management, respectively. Delivering these agents locally via PLA microspheres can achieve high site-specific concentrations with reduced systemic exposure [4,5]. However, even biocompatible polymers like PLA can exhibit cytotoxic or haemolytic effects depending on degradation kinetics, residual solvents, or drug–polymer interactions [6]. Without rigorous biological testing, such risks remain poorly characterized.

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The use of established cell line models for in vitro cytotoxicity studies provides a controlled, reproducible, and ethically sound means to assess cellular responses before in vivo evaluation. The MTT assay, in particular, quantifies metabolic activity as an indicator of cell viability and proliferation, enabling the detection of subtle cytotoxic effects that may not be apparent macroscopically [7,8]. By selecting cell lines relevant to oral wound healing, it is possible to model how the microspheres might influence early regenerative events within extraction sockets.

Equally important is hemocompatibility testing. Intra-socket materials are in immediate contact with blood, and any significant interaction with red blood cells (RBCs) could trigger hemolysis, impair clot stability, and delay healing. Hemolysis assays provide a direct measure of RBC membrane integrity upon exposure to the biomaterial. International standards such as ASTM F756-17 and ISO 10993-4 set thresholds (<5% hemolysis) for acceptable hemocompatibility, ensuring that materials do not compromise blood function during clinical use [9,10].

This study addresses a critical gap in the preclinical evaluation of Amoxicillin- and Diclofenac Sodium-loaded PLA microspheres for intra-oral drug delivery. By combining MTT-based cytotoxicity assays on relevant cell lines with standardized hemocompatibility testing, the work aims to generate a comprehensive safety profile for these formulations. Such data are essential not only for confirming their suitability for post-extraction use but also for guiding formulation optimization to maximize therapeutic benefit while minimizing biological risk.

2. Materials and Methods

Safety and biocompatibility of the microspheres were evaluated using two critical biological assays: haemolysis and cell viability studies.

Hemolysis Assay

The hemocompatibility was evaluated via hemolysis assay. Fresh human blood was collected in anticoagulant-containing tubes, centrifuged to separate erythrocytes, and washed thrice in sterile saline. Erythrocyte suspension (2%) was prepared by dilution with saline. Microspheres at specified concentrations were incubated with erythrocytes at 37°C for one hour. After incubation, samples were centrifuged, and supernatants analyzed spectrophotometrically at 540 nm. Percentage hemolysis was calculated using saline as a negative control (0% hemolysis) and distilled water as a positive control (100% hemolysis).

Cell Line Studies (MTT Assay)

Cell viability was assessed using the MTT assay. Standard fibroblast cell lines were cultured in 96-well plates with complete growth medium and incubated overnight at 37°C and 5% CO_2. Microsphere samples (10, 25, 50, and 100 µg/ml) were added to wells and incubated for 24 hours. Post-incubation, MTT solution (5 mg/ml) was introduced and cells incubated for an additional 4 hours, allowing viable cells to convert MTT into insoluble formazan crystals. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells, verifying microsphere cytocompatibility at therapeutic concentrations.

3. Results

Hemolysis Assay

Hemolysis Assay was performed for both formulations to assess biocompatibility. (Figure 1)

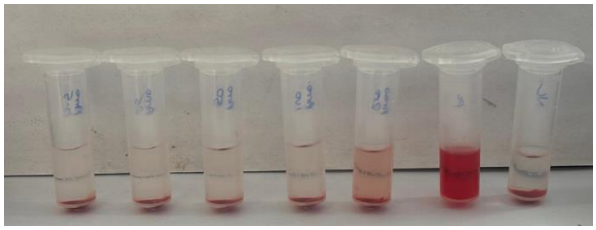

Figure 1. Hemolysis Assay of Amoxicillin loaded Poly-lactic acid microspheres

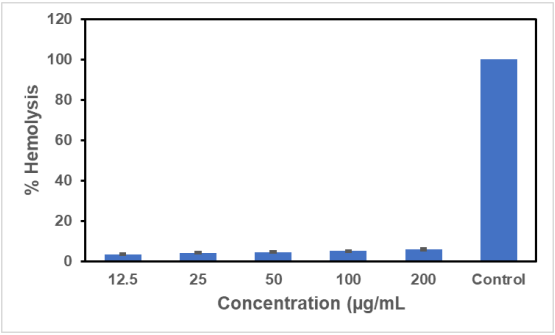

Figure 2. Graph portraying Hemolytic Assay for Amoxicillin loaded Poly-lactic Acid microspsheres.

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Figure 2 shows the hemolytic assay data for amoxicillin-loaded PLA microspheres demonstrate minimal hemolysis across all tested concentrations. The values are as follows:

- 12.5 µg/ml: 3.64%
- 25 µg/ml: 4.31%
- 50 µg/ml: 4.70%
- 100 µg/ml: 5.18%
- 200 µg/ml: 6.01%
- Control (positive lysis): 100%

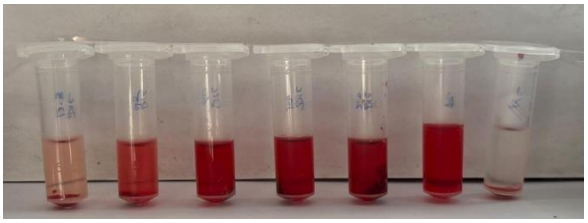

Figure 3. Hemolysis Assay of Diclofenac Sodium loaded Poly-lactic acid microspheres

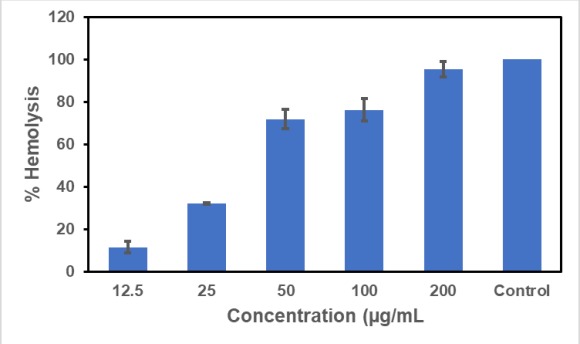

Figure 4. Graph portraying Hemolytic Assay for Diclofenac Sodium loaded Poly-lactic Acid microspheres.

The hemolytic response to diclofenac sodium-loaded PLA microspheres shows a concentration-dependent increase in hemolysis, as seen in Figure 36 and Figure 37.

- 12.5 µg/ml: 11.54%
- 25 µg/ml: 32.17%
- 50 µg/ml: 71.98%
- 100 µg/ml: 76.21%
- 200 µg/ml: 95.30%
- Control (positive lysis): 100%

Cell Line Studies (MTT Assay)

MTT assay results assessed microsphere cytotoxicity using standard fibroblast cell lines. (3T3 cell lines). Both drug-loaded microsphere formulations demonstrated high cell viability across all tested concentrations (500µg/ml and 125µg/ml) at the end of three days. The cell morphology is seen in Figure 5 and figure 7.

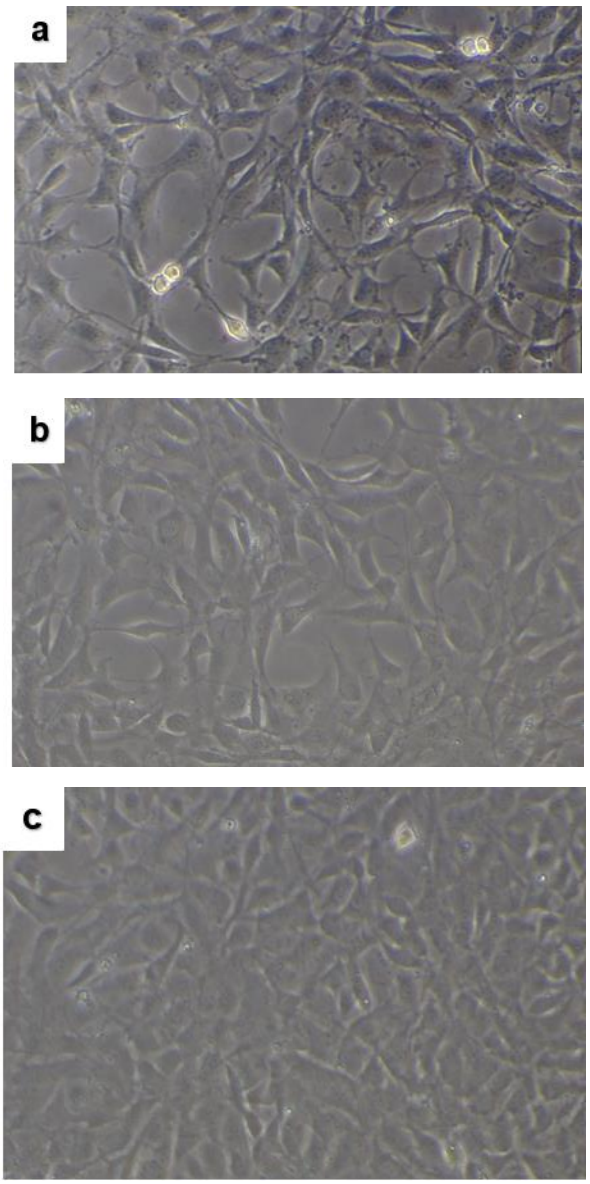

Figure 5. Morphology of 3T3 treated with (a) 500µg and (b)125µg of Amoxicillin loaded PLA microspheres (c)control

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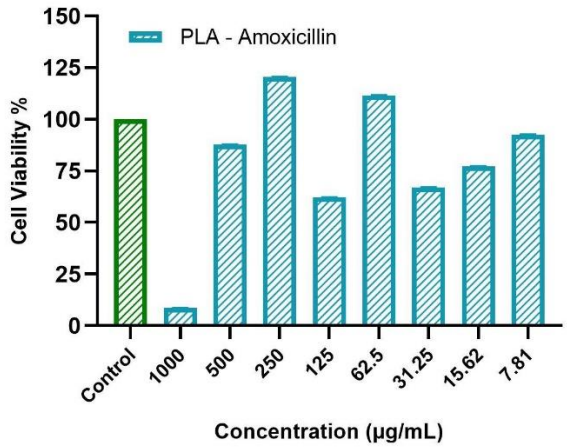
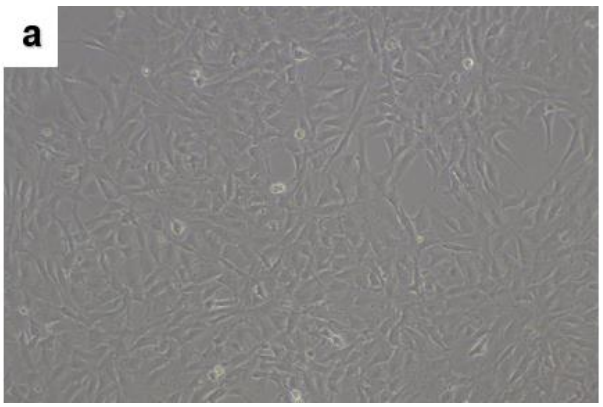


Figure 6. Percentage of viable fibroblasts at the end of three days

Figure 6 shows the viability profile for amoxicillin-loaded PLA microspheres also exhibits a concentration-dependent trend. The highest concentration (1000 µg/mL) leads to significant cytotoxicity with viability reduced to 8.7%. Contrastingly, at 500 µg/mL, the viability sharply increases to 87.7%, demonstrating minimal toxicity. Notably, an unusually high viability exceeding 100% (120.6% at 250 µg/mL and 111.6% at 62.5 µg/mL) indicates possible cell proliferation or metabolic stimulation by amoxicillin at these doses. Lower concentrations show a good biocompatibility profile (125 µg/mL - 62.3%, 31.25 µg/mL - 67.0%, 15.62 µg/mL - 77.3%, and 7.81 µg/mL - 92.6%). Overall, amoxicillin-loaded PLA microspheres demonstrate optimal biocompatibility and potential cell proliferative effects particularly at intermediate concentrations (250 µg/mL and 62.5 µg/mL), though cytotoxicity is evident at higher concentrations (≥1000 µg/mL).



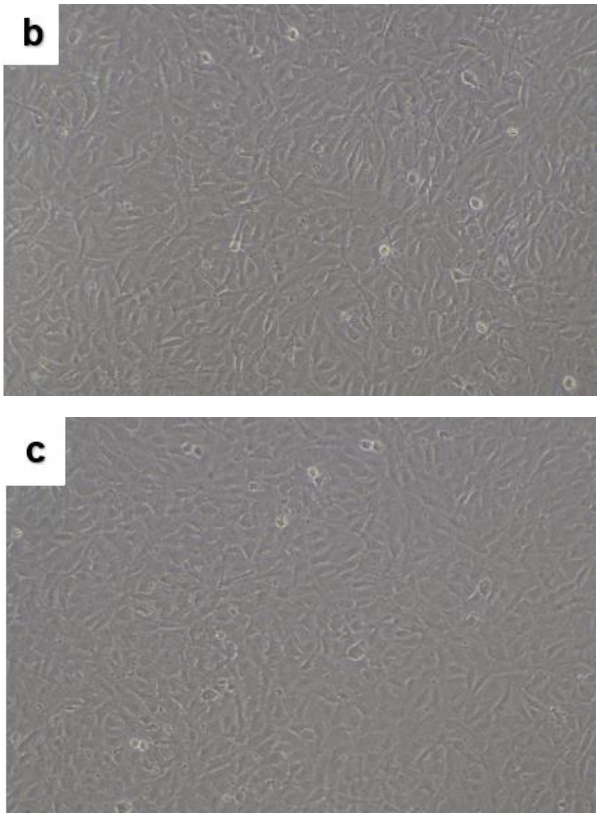


Figure 7. Morphology of 3T3 treated with (a) 500µg and (b)125µg of Diclofenac Sodium loaded PLA microspheres (c) control

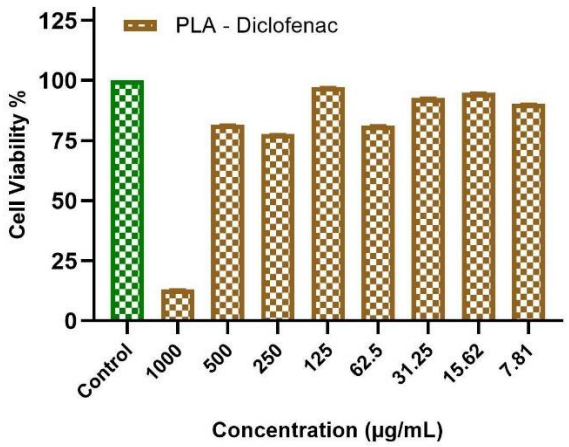


Figure 8. Percentage of viable fibroblasts at the end of three days

The cell viability observed with diclofenac-loaded PLA microspheres shows a concentration-dependent relationship. (Figure 41) At the highest concentration

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(1000 µg/mL), cell viability is significantly low (13.1%), suggesting a marked cytotoxic effect at this dosage. However, viability dramatically improves at lower concentrations, with 500 µg/mL showing 81.6% and 250 µg/mL showing 77.7%. Interestingly, the best cell viability is observed at intermediate (125 µg/mL, 97.3%) and lower concentrations (62.5 µg/mL - 81.3%, 31.25 µg/mL - 92.8%, 15.62 µg/mL - 94.9%, and 7.81 µg/mL - 90.3%), indicating optimal biocompatibility at these concentrations. Therefore, diclofenac-loaded PLA microspheres are biocompatible and safe for cells at concentrations below 250 µg/mL, but high concentrations above this threshold may exert substantial cytotoxicity.

4. Discussion

This study assessed the biocompatibility of diclofenac sodium–loaded PLA microspheres using MTT and hemolysis assays. MTT results demonstrated over 90% fibroblast viability at concentrations up to 125 µg/mL, indicating favorable cytocompatibility. This aligns with previous findings that PLA-based microspheres degrade into lactic acid, which is naturally metabolized and well tolerated by soft tissues [11]. In contrast, the hemolysis assay revealed significant erythrocyte lysis at higher concentrations, exceeding the 5% hemolysis threshold set by ISO 10993-4. Hemolysis peaked above 90% at 200 µg/mL, likely due to membrane-disruptive effects of diclofenac when not fully encapsulated [12]. The discrepancy between fibroblast viability and hemolytic activity highlights the importance of dose optimization and encapsulation efficiency. Prior studies have emphasized that drug-polymer ratio, particle size, and surface characteristics critically influence drug release and biocompatibility [13]. Overall, while the formulation appears safe for local intraoral use at lower concentrations, further modifications—such as surface coating or PEGylation—may enhance blood compatibility and reduce hemolytic risk [14].

5. Conclusion

This study demonstrated that diclofenac sodium–loaded PLA microspheres exhibit favorable cytocompatibility in fibroblast cultures at concentrations up to 125 µg/mL, indicating their potential for safe intraoral application in soft tissue environments such as extraction sockets. However, a marked increase in hemolytic activity at higher concentrations underscores the importance of controlled dosing and formulation optimization to minimize blood-contact cytotoxicity. The results suggest that while the microspheres are biocompatible with oral fibroblasts, caution must be exercised to avoid systemic exposure or high local concentrations that could compromise erythrocyte integrity. Future improvements, such as surface modification or co-polymer blending, may enhance the safety profile and broaden the clinical applicability of this localized drug delivery system.

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