In The Name of Allah, The Most Beneficent and The Most Merciful

KULLIYYAH OF PHARMACY

FAST TALK #9/2017

FUSION CONCEPT BETWEEN CONTEMPORARY MEDICINE AND NATURAL PRODUCTS: A NEW PARADIGM IN THE DESIGN OF SAFER MEDICINE

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OUTLINE

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- Introduction
- Potential solution
- Characterisation, in-vitro and in-vivo data
- Conclusion

Introduction

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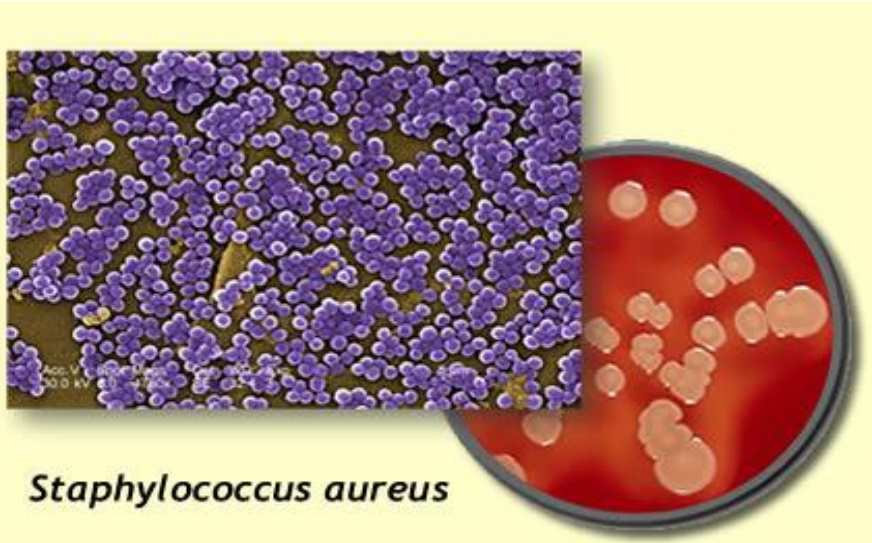
Osteomyelitis:

- **bone infection**
- **causes: local injury, haematogenous spread or prostheses-related**
- pathogen: mainly **Staphylococcus bacteria**
- difficult to treat especially if prostheses is involved as bacteria becomes biofilm-type



Conventional Treatment

Poly (Methyl Methacrylate) (PMMA) Beads Containing Gentamicin (Septopal):

- contains 4.5 mg of gentamicin
- 7 mm in diameter
- each chain carrying 30 beads

Concern with Conventional Treatment

- High cost :around USD 15K to 30 K primarily just for the implants
- Poor patient management.
- Need to undergo multiple surgical procedures.
- Relatively short period of time for extended drug release (10-14 days)
- Non-biodegradable materials.

Potential Solutions: New beads

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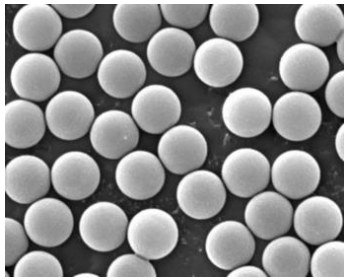
- Longer, extended release.
- Improved patient management & care.
- Overcome the need for multiple surgical procedures.
- Biodegradable and biocompatible materials.

PLGA microspheres loaded with gentamicin and *N. sativa* (NSO)



Inspiration on selection of Habbatus Sawda

- Al-Bukhari: Related by Aishah (Allah bless her) that the Prophet had said: the black seed cures everything except death.

Contemporary medicine

+

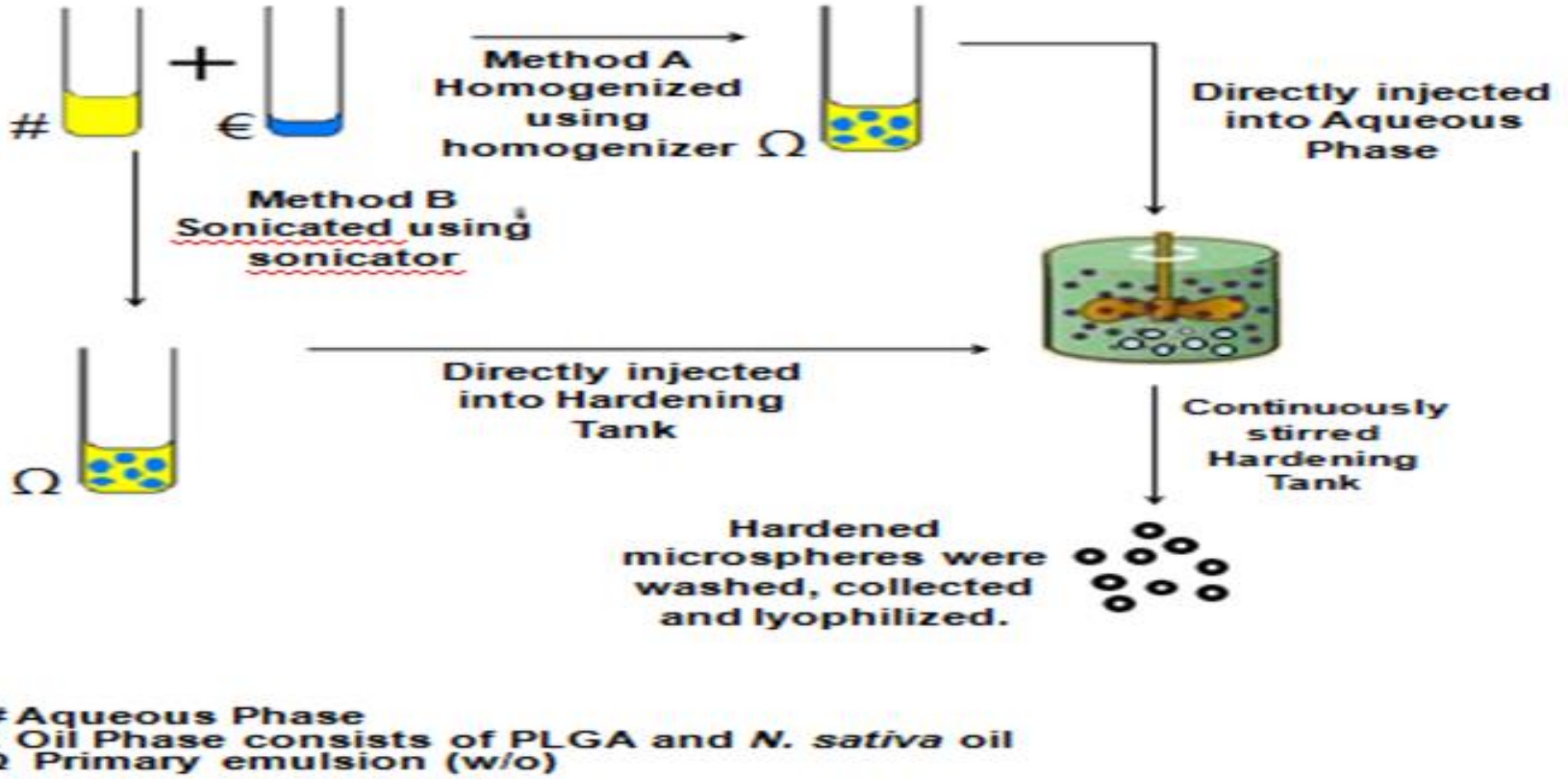
Prophetic medicine

Gentamicin

+

Nigella sativa oil

Fabrication Procedures



Method A for fabricating gentamicin loaded PLGA microspheres
Method B for fabricating NSO loaded PLGA microspheres

Formulations

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- 27 formulations for both gentamicin and NSO microspheres.
- Gentamicin microspheres:
  - Different blends of PLGA (Intrinsic Viscosity 0.2, 0.4 and 1.0 dL/g).
  - Ratio for the PLGA blends (25:75, 75:25 and 50:50)
  - Surfactants used (TX 100, Tween 80, PVA).
- NSO microspheres:
  - Different blends of PLGA (Intrinsic Viscosity 0.2, 0.4 and 1.0 dL/g).
  - Ratio for the PLGA blends (25:75, 75:25 and 50:50)
  - Amount of NSO loaded (50, 75 and 100 mg).

OUTCOMES

Size Distribution & Zeta Potential

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- For gentamicin loaded microspheres:
  - size distribution range from 463.67 nm ± 52.54 to 4602 nm ± 113.58
  - zeta potential ranging from -5.40 mV ± 0.62 to -21.27 mV ± 0.91.

- For NSO loaded microspheres:
  - size distribution ranging from 409.67 nm ± 37.45 to 6568.00 nm ± 147.22
  - zeta potential between -6.80 mV ± 1.65 to -17.43 mV ± 1.85.

Drug Loading for Gentamicin-loaded

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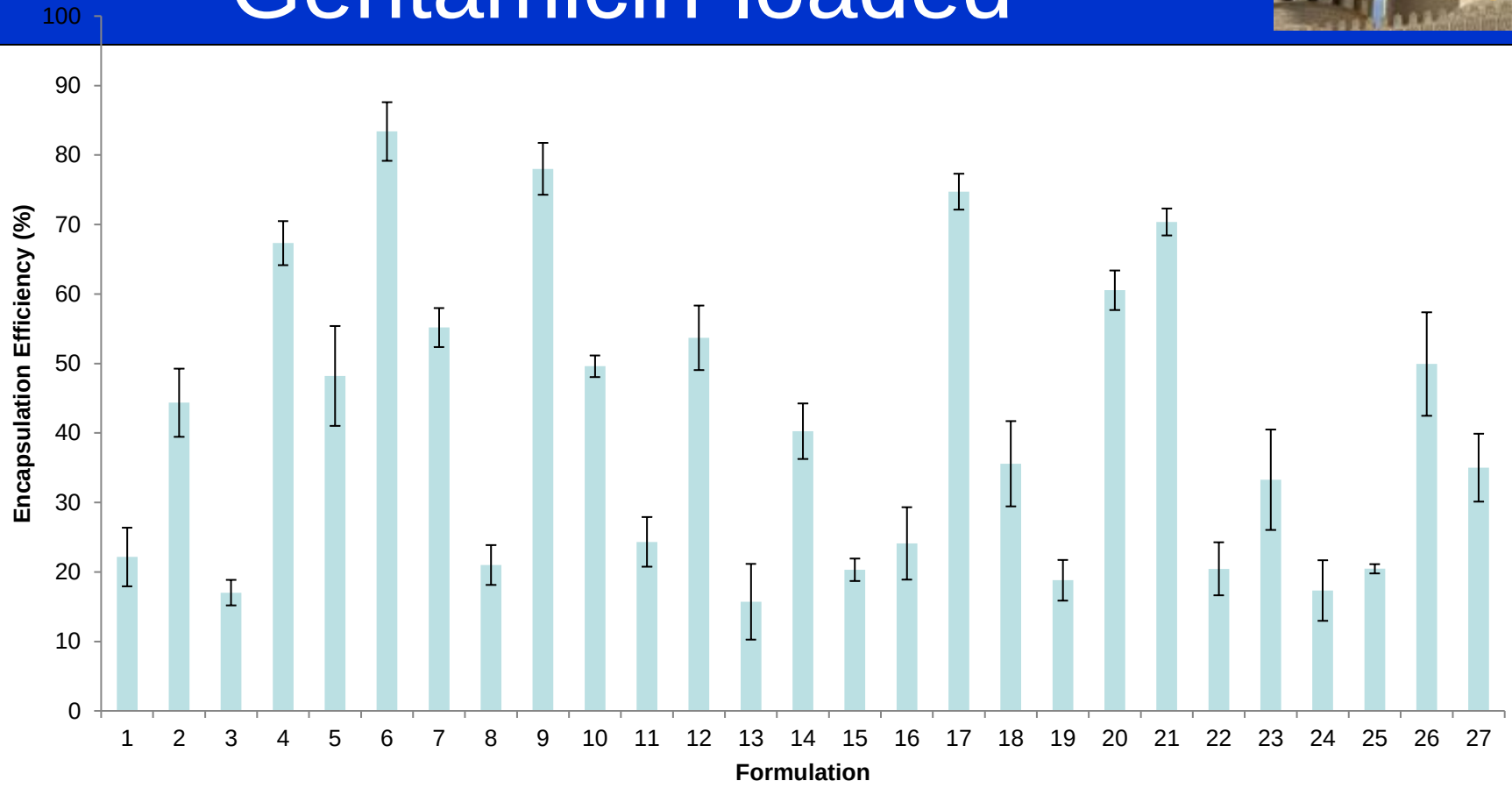

Five highest drug loading efficiency:

1. Formulation 4 (67.32% ± 3.18)
2. Formulation 6 (83.39% ± 4.22)
3. Formulation 9 (78.01 ± 3.73)
4. Formulation 17 (74.72% ± 2.58)
5. Formulation 21 (70.38% ± 1.94)

Drug Loading for NSO-loaded Microspheres

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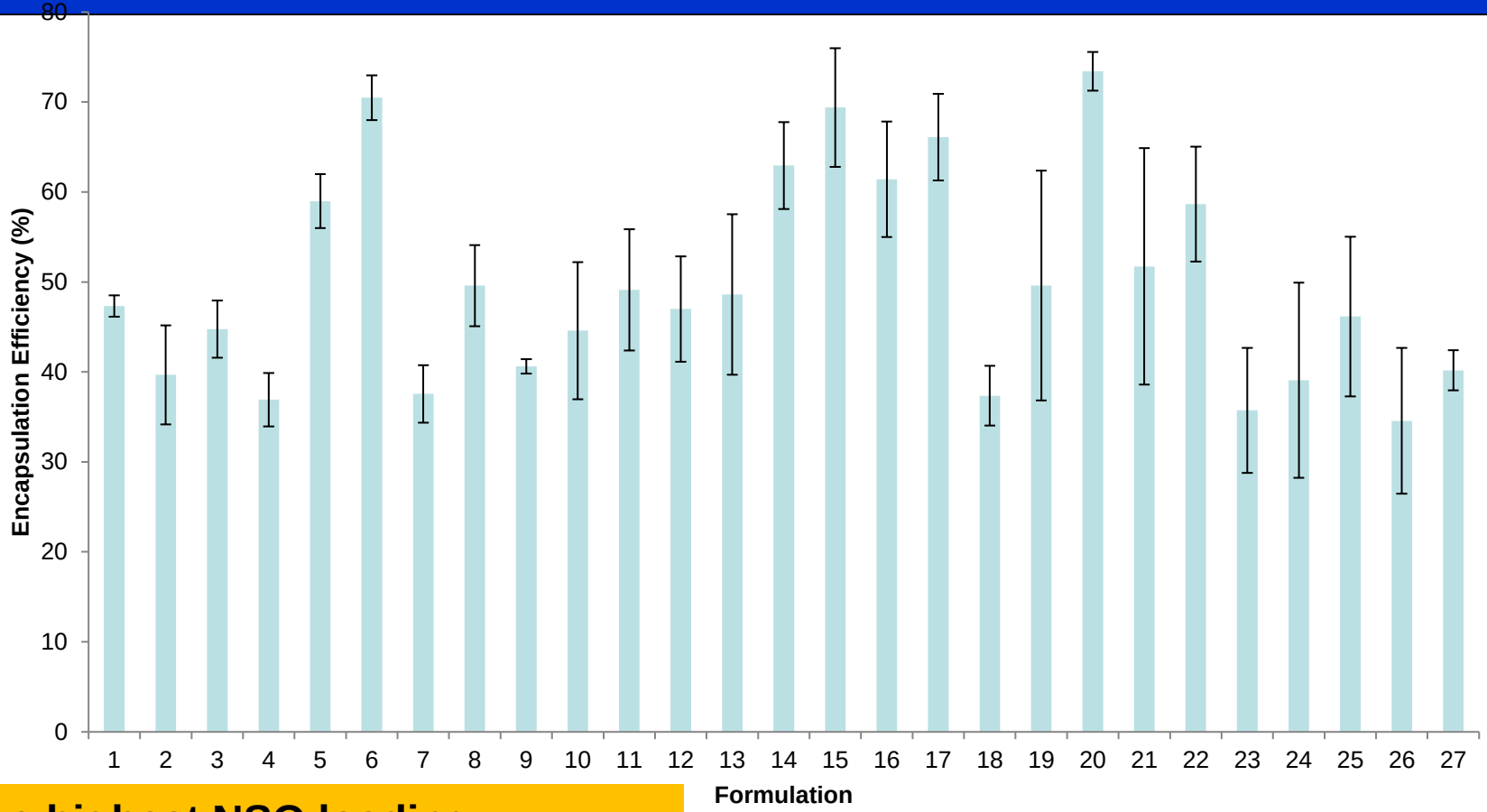
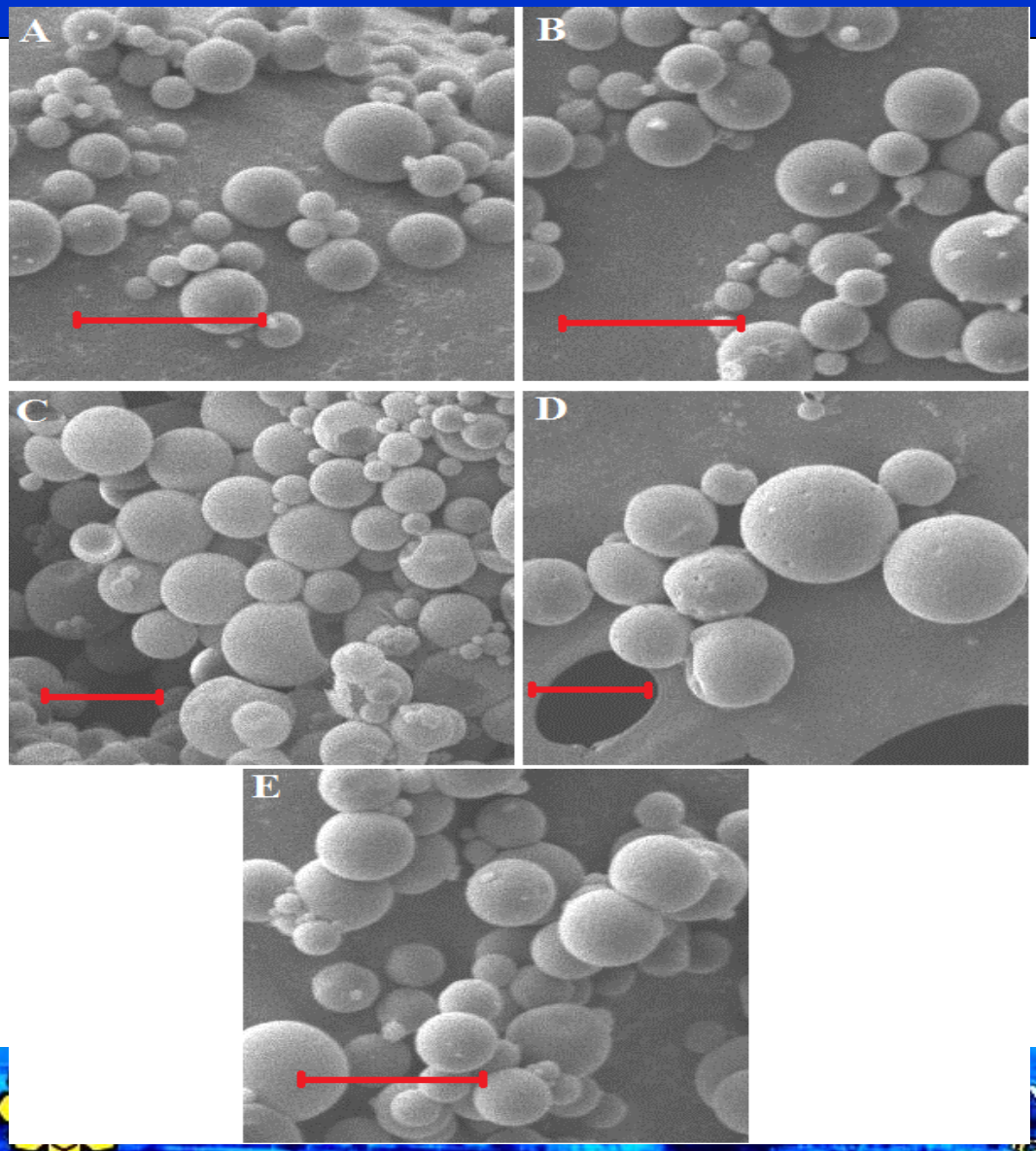


Five highest NSO loading efficiency:

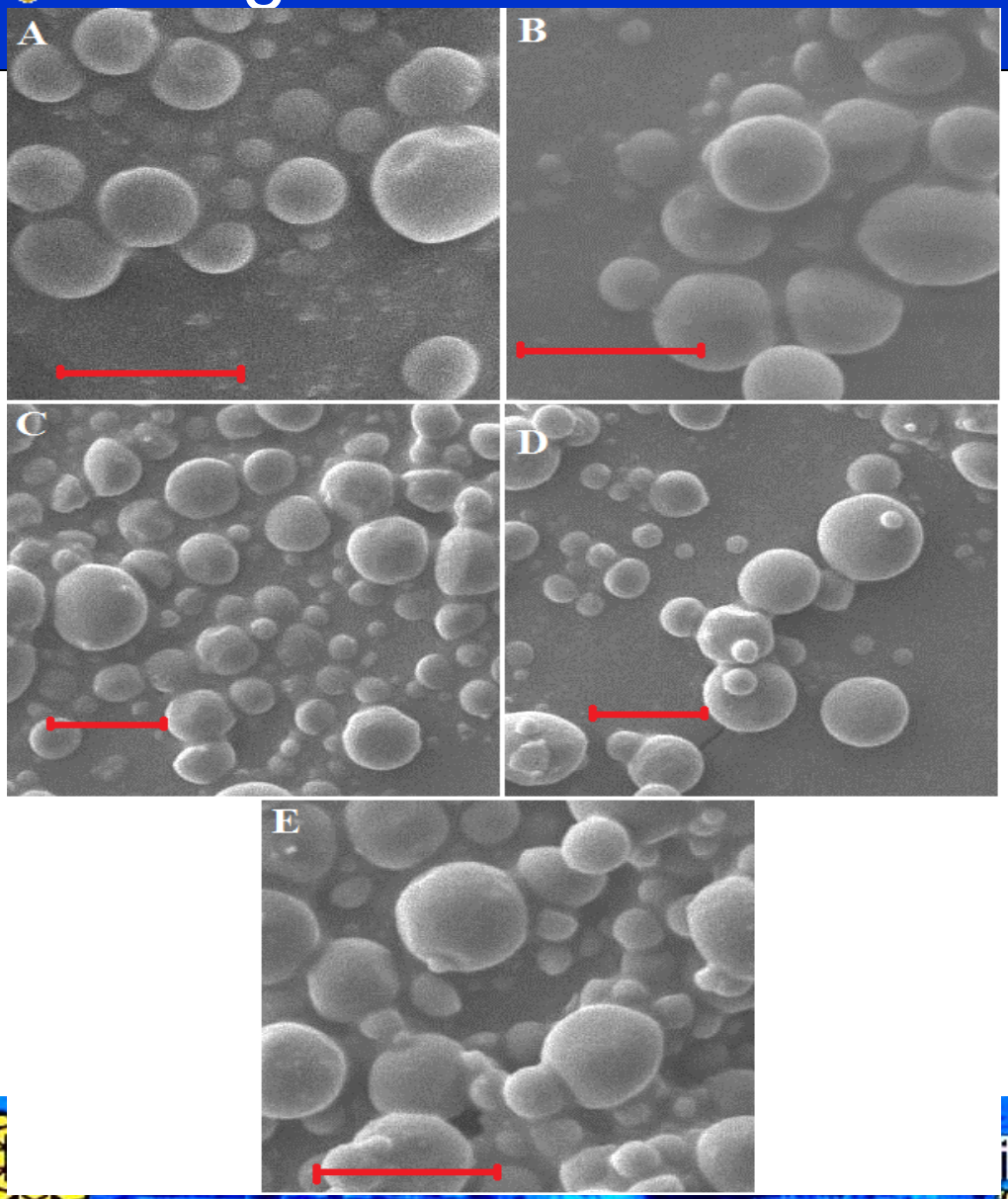
1. Formulation 6 (70.48% ± 2.49)
2. Formulation 14 (62.94% ± 4.84)
3. Formulation 15 (69.39% ± 6.60)
4. Formulation 17 (66.11% ± 4.81)
5. Formulation 20 (73.42% ± 2.14)

External Morphology of Highest Gentamicin-Load Microspheres



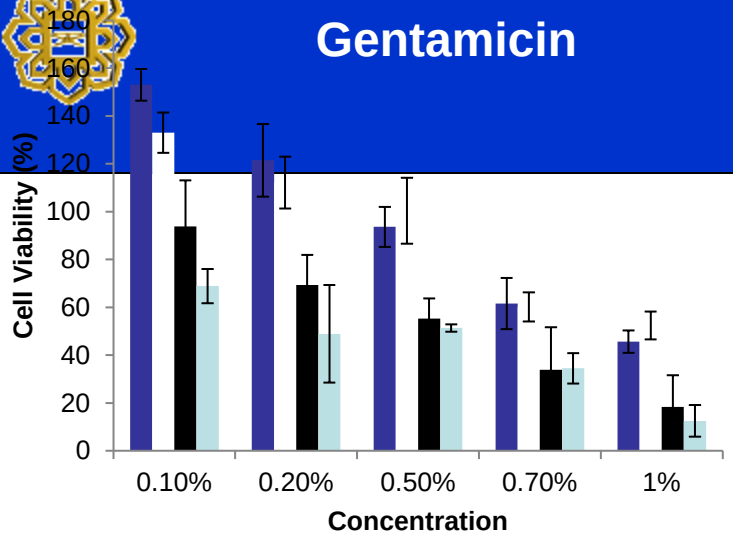
SEM images for Formulation 4, 6, 9, 17 and 21 (Figure A, B, C, D and E respectively, showing well-rounded particles.
(Scale bars : 10 µm.)

External Morphology of Highest NSO-Load microspheres

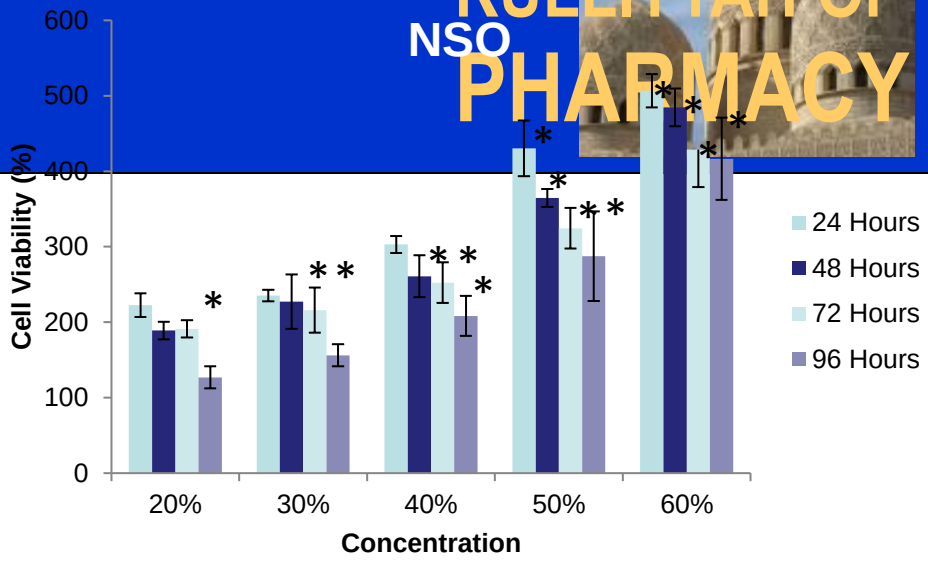


SEM images of NSO loaded microspheres for formulation 6, 14, 15, 17 and 20 (Figure A, B, C, D and E respectively showing well-rounded particles. (Scale bars : 10 µm.)

Gentamicin



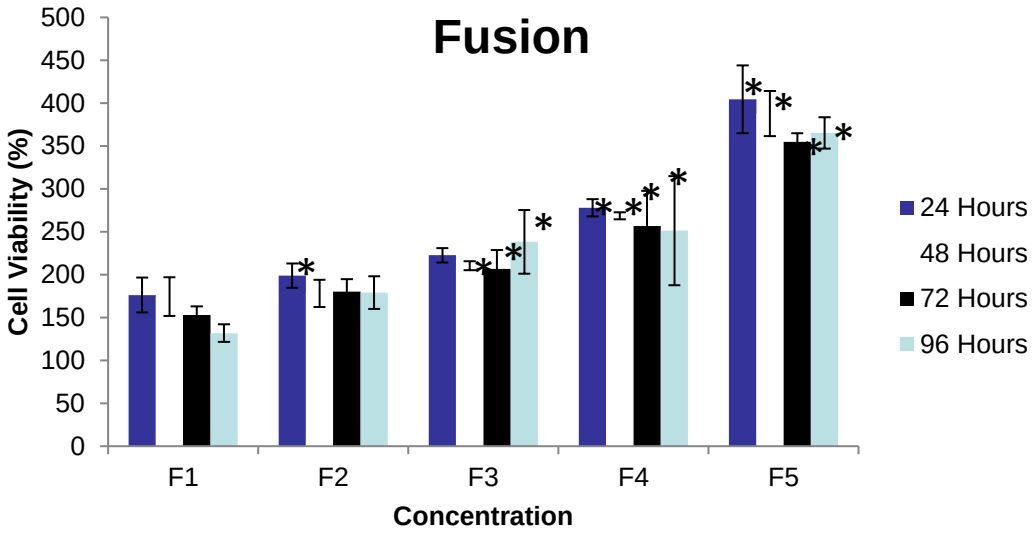
NSO



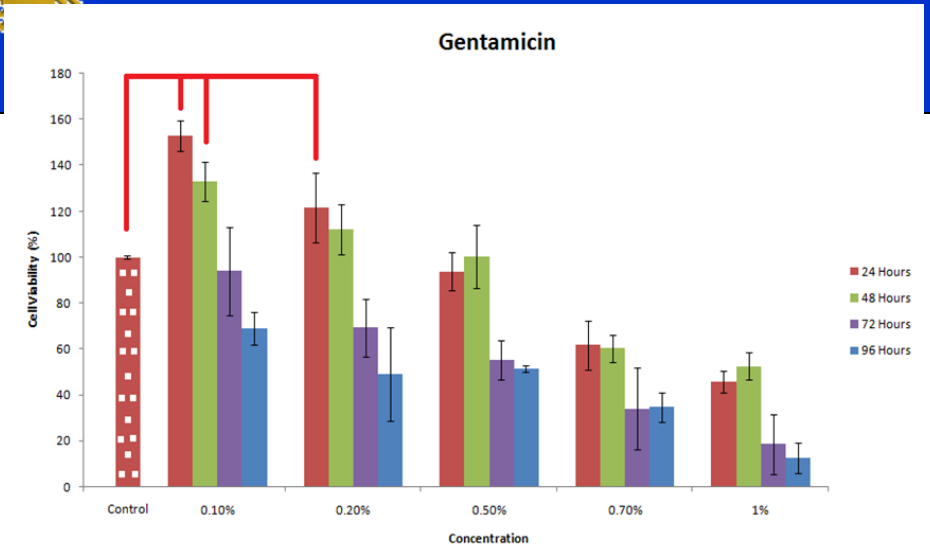
Cell viability study (one-way ANOVA, Tukey's test, $p < 0.05$) showing:

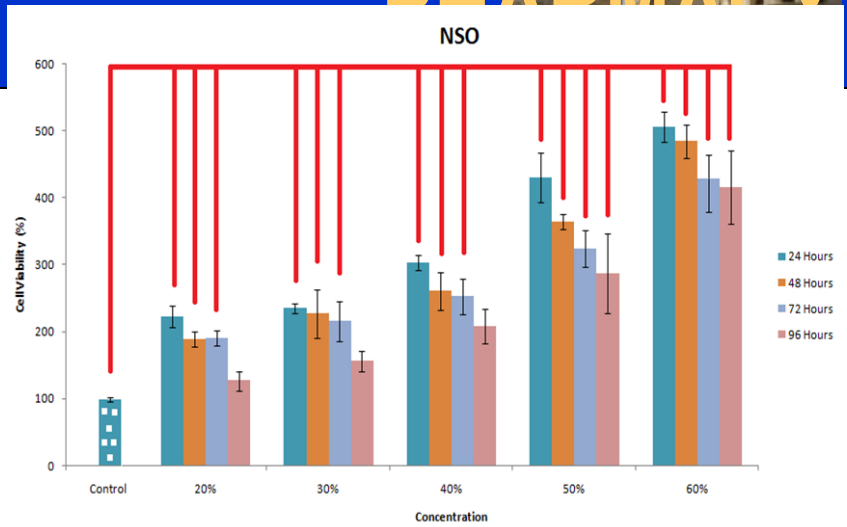
1. Lower [gentamicin] = higher viability
2. Higher [NSO] = higher viability
3. F5 fusion (high [NSO], high [gentamicin] = higher viability

Fusion

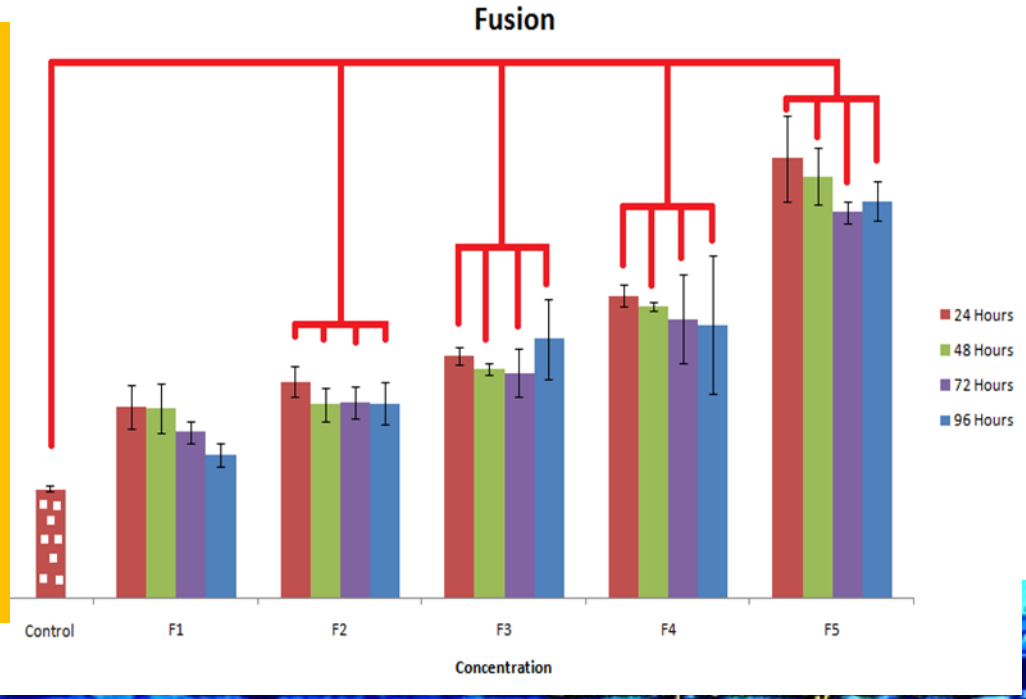


Cell viability of gentamicin, NSO and fusion





-Lower [gentamicin] = higher viability
-Higher [NSO] = higher viability
-F5 fusion (high [NSO], high [gentamicin] = higher viability

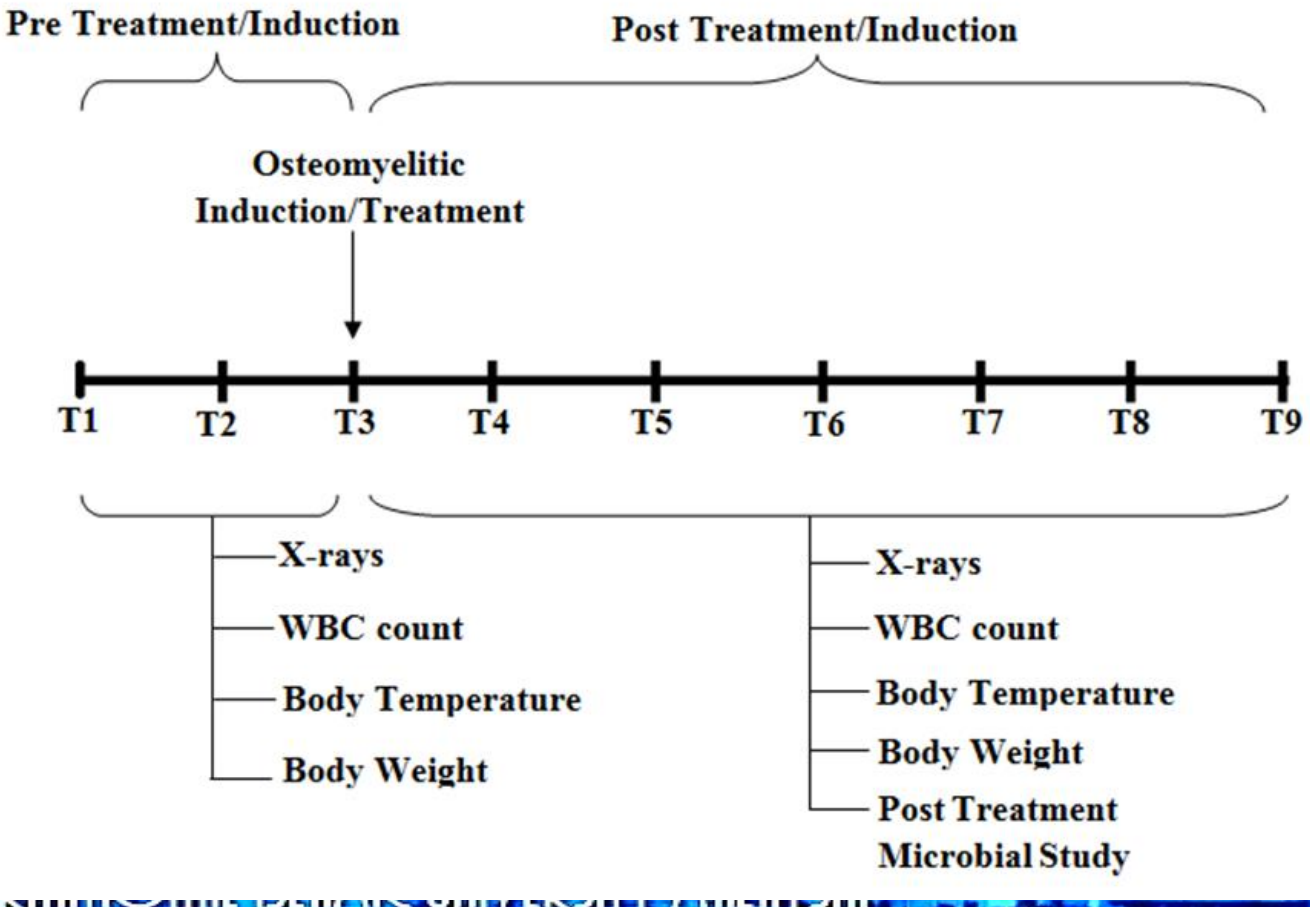


Fusion show safer profile than commercial Gentamicin beads

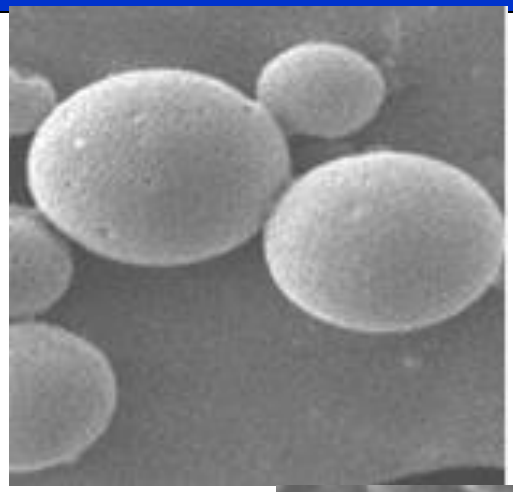
Nigella sativa + Gentamicin disk

Commercial gentamicin beads

Time line for the overall flow of the animal study showing the main evaluations and data collection events. The events were carried out based on the assigned weeks as denoted with T in the time line.

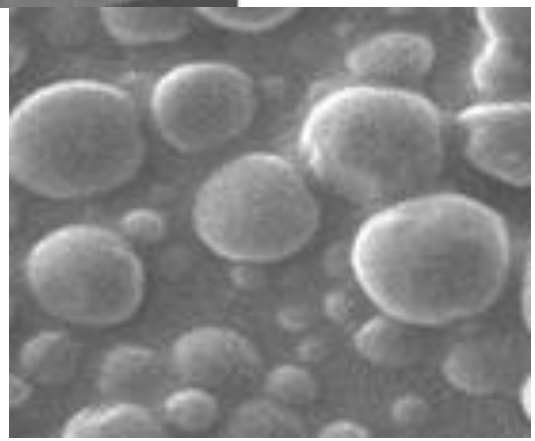


SEM images of microsphere

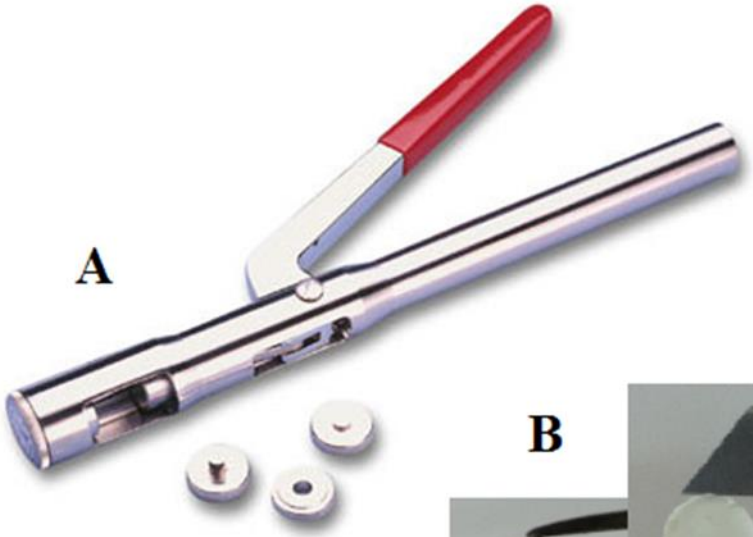


Gentamicin MS

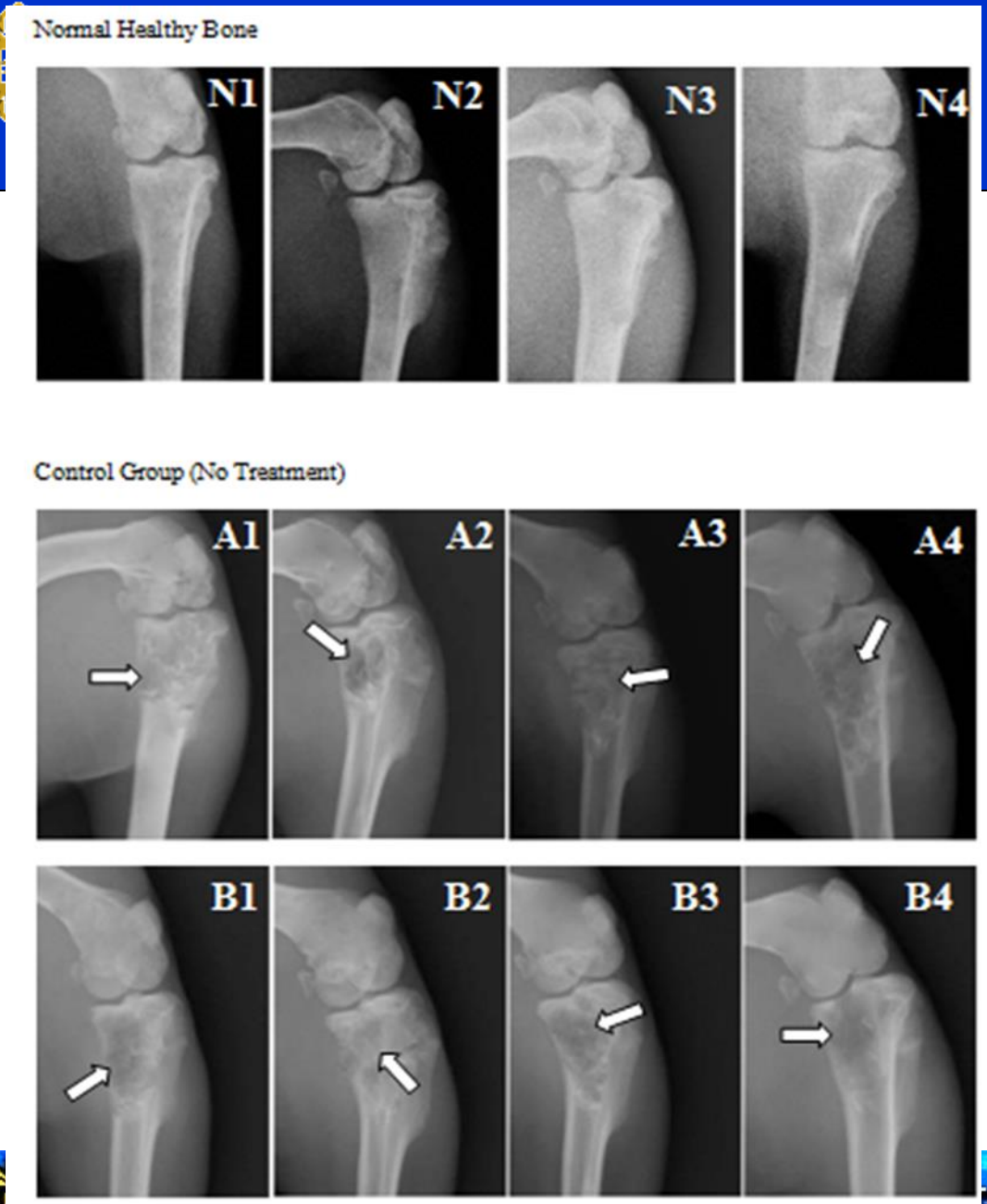
NSO MS



The Quick Press Pallet Kit (A) and the shape of the compressed NSO+genta microspheres (B).

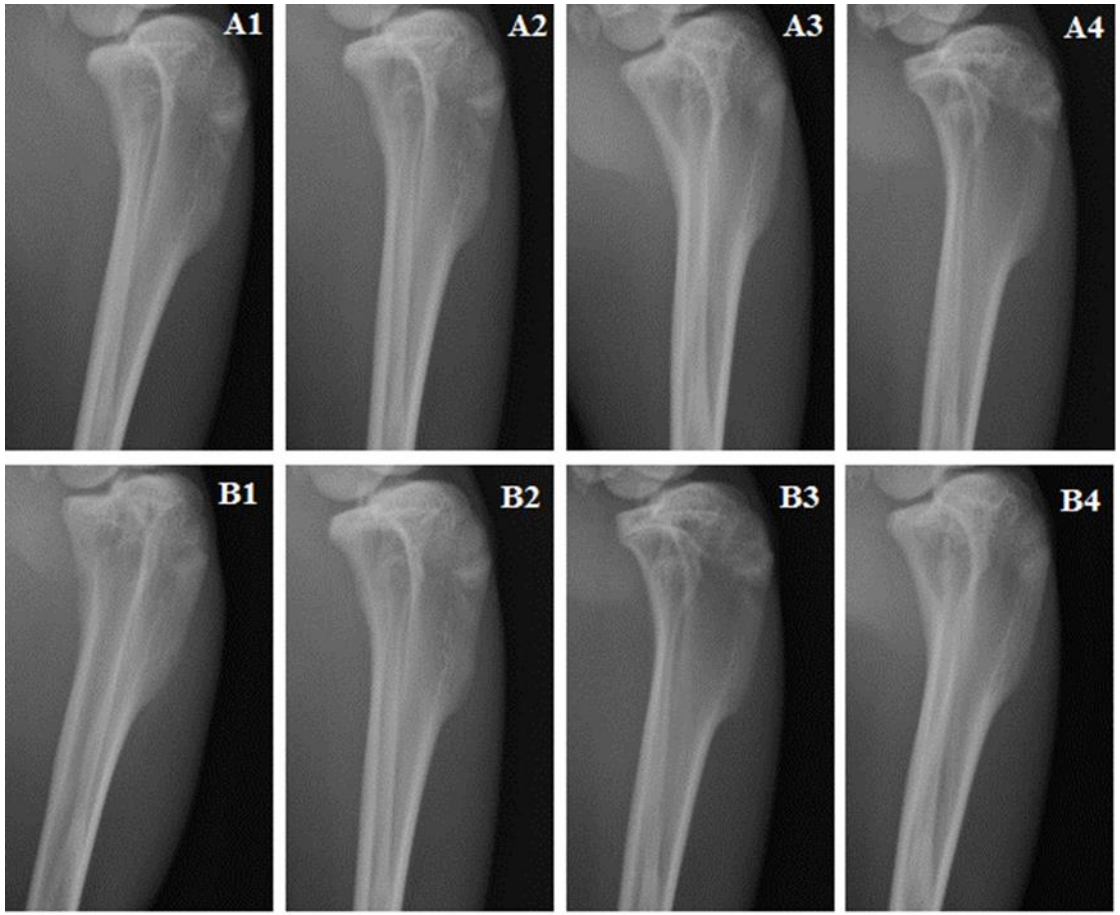


X-ray results – pre-treatment



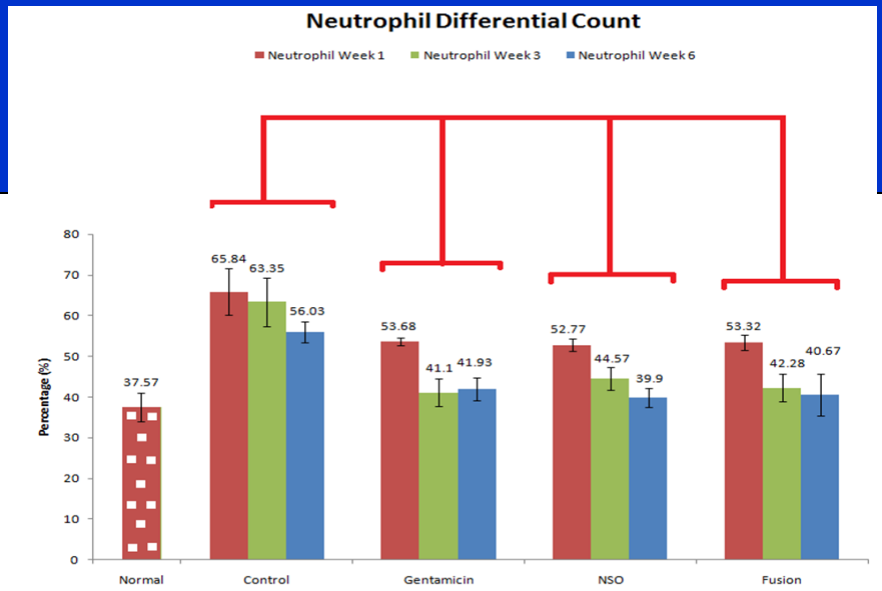
Arrow indicate radiolucency of proximal tibiae, area that inoculated with *S.Aureus*

Treatment group

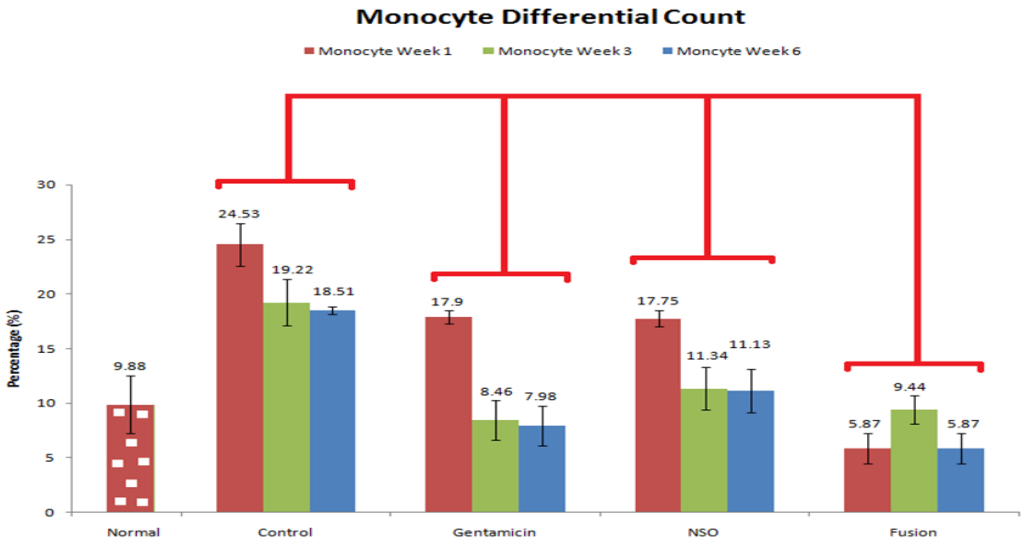


X-ray results – post treatment

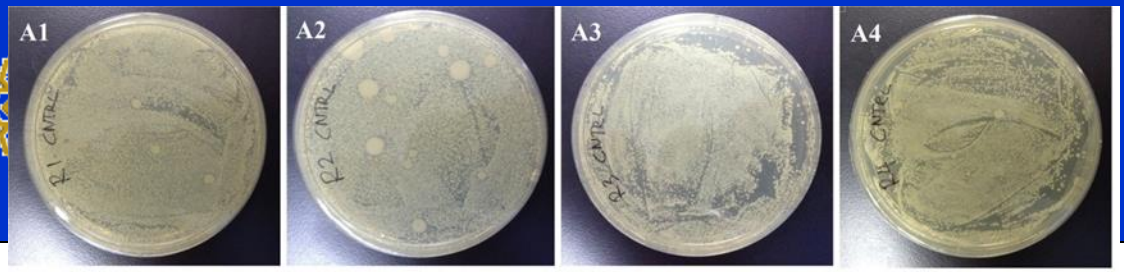
Taken at week 3 and 6; showing no sign of demarcation

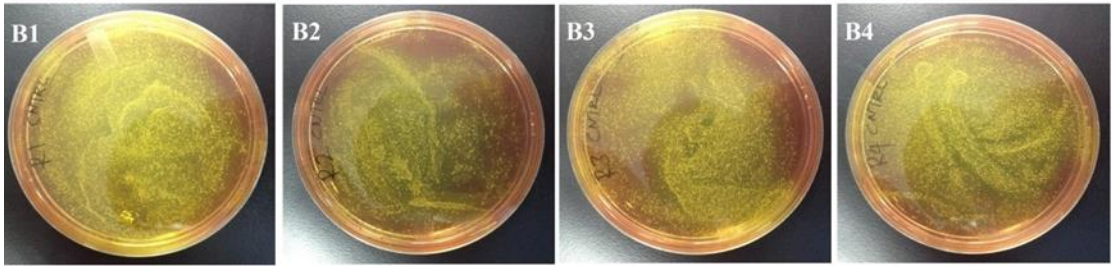


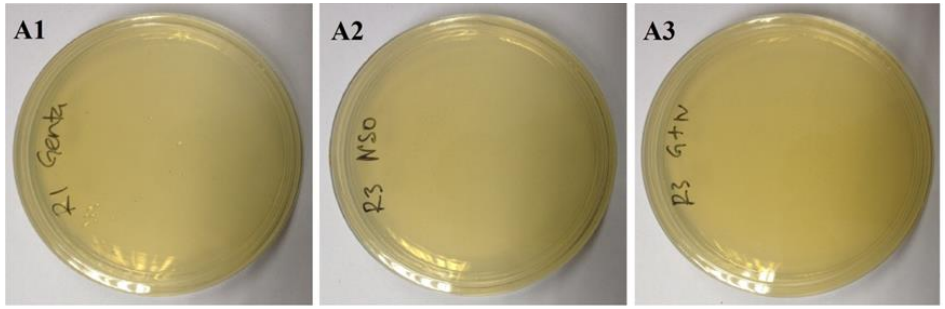
WBC results



Fusion showed better profile of WBC







Post surgical results

Control gp (above) showed bacterial colonies; treatment gp (below) clear from bacterial growth

In-vitro Release Profile

- Only five formulations with the highest drug loadings were chosen from gentamicin and NSO microspheres.
- 30 mg of microspheres were utilized for each group.
  - Comparison between powdered and compressed microspheres was made.
    - Compressed microspheres have 15% (4.5 mg) extra kaolin as a filler.

Gentamicin Loaded Microspheres

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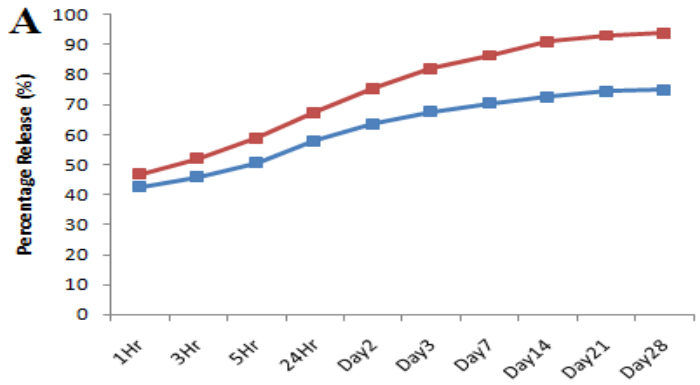

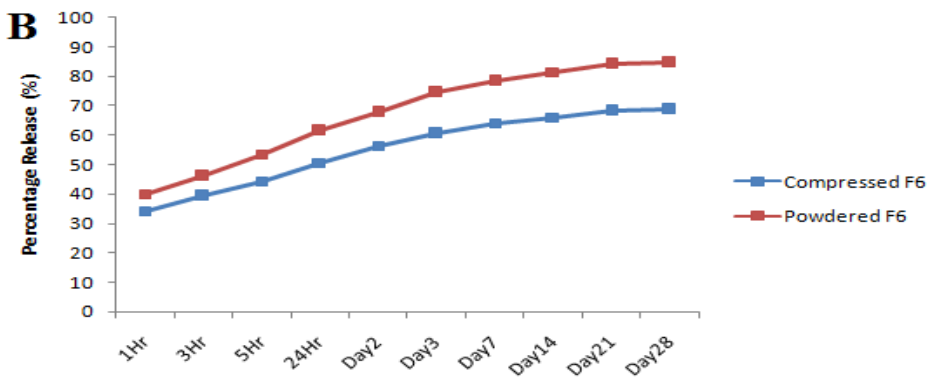

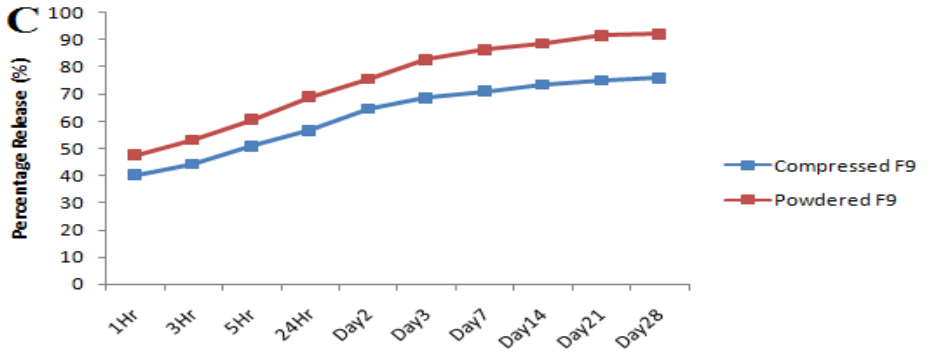

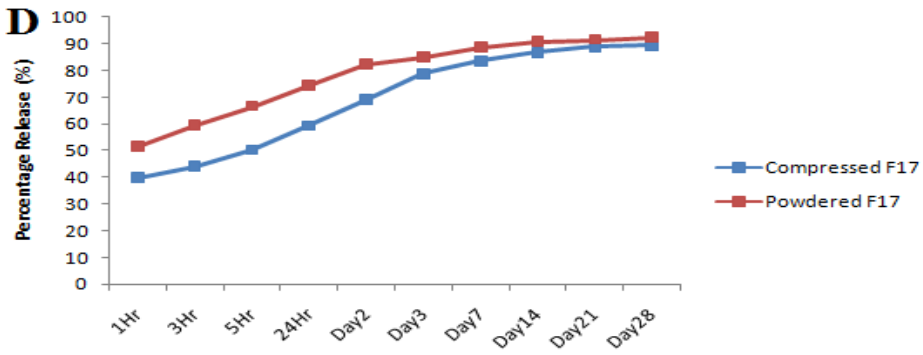

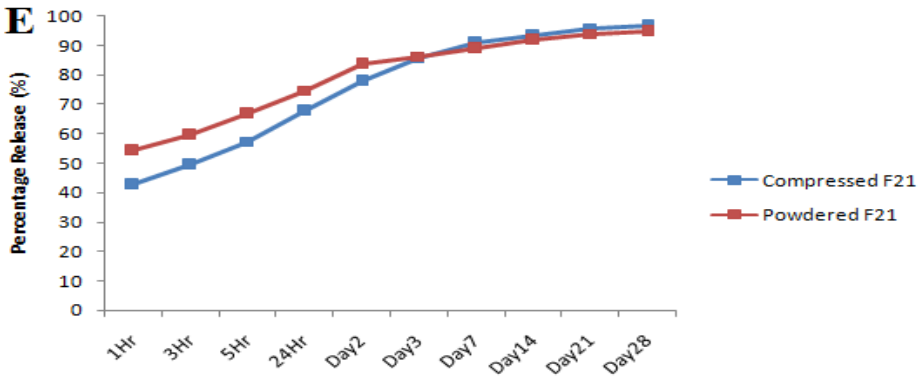

In-vitro Release Profile

- All five gentamicin formulations (compressed and powdered microspheres) showed >30% initial release of gentamicin
- higher amount of lower intrinsic viscosity of PLGA used = higher the initial release at 1st hour (see formulation 17 & 21)
  - possibly faster degradation of polymer.
- In general, powdered microspheres has a slightly higher release rate than compressed

NSO Loaded Microspheres

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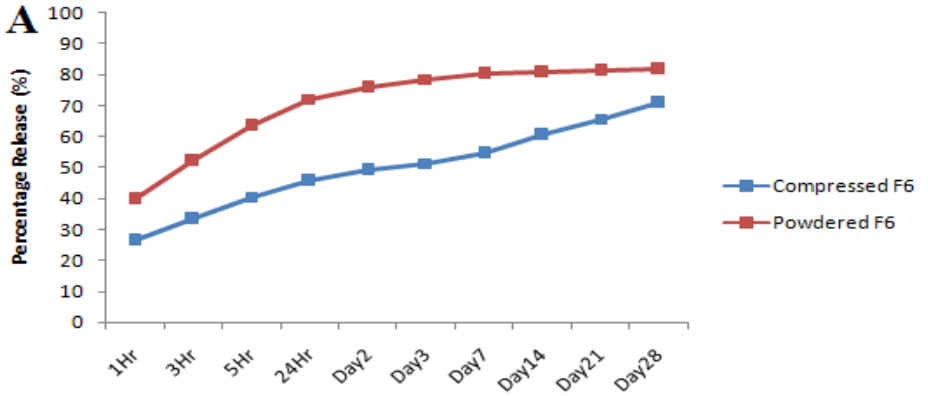

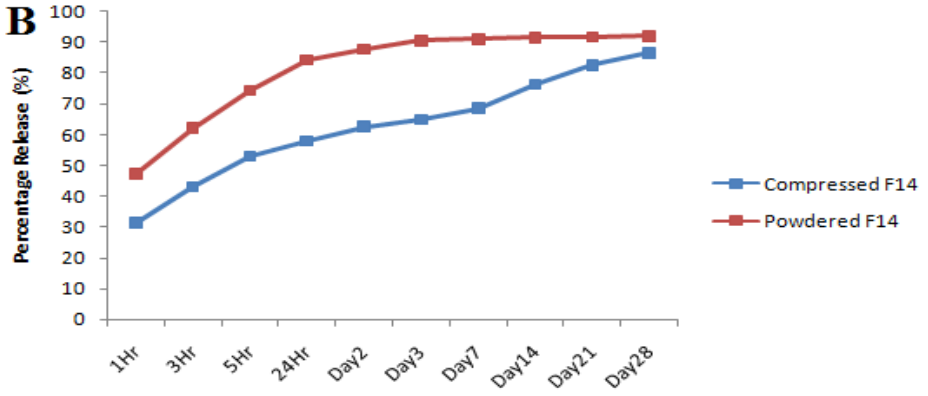

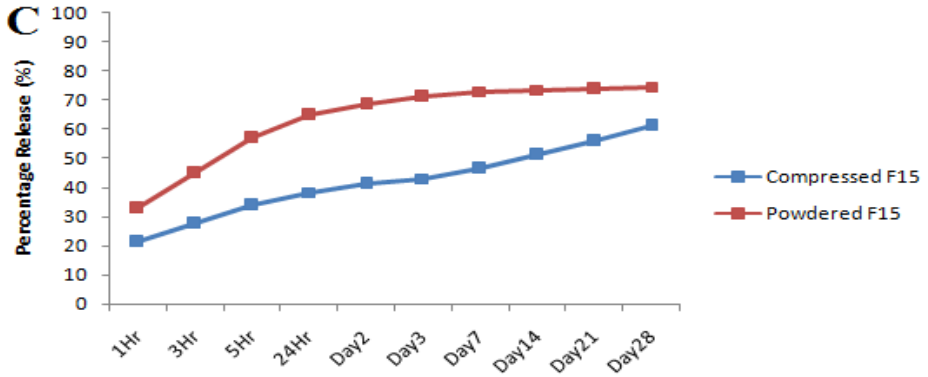

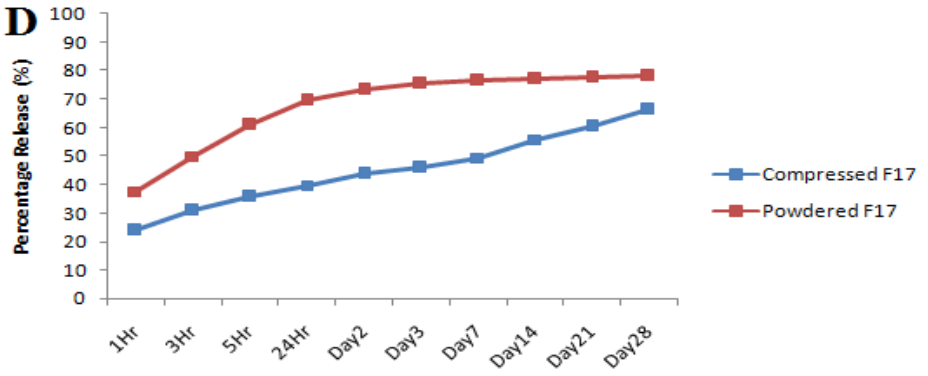

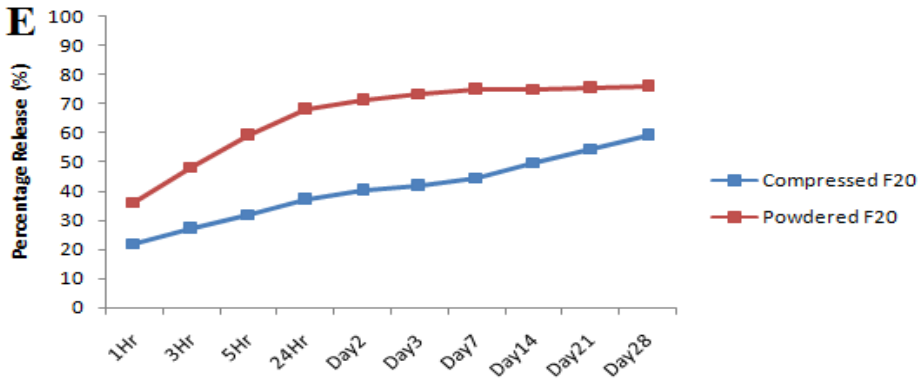

In-vitro Release Profile

- NSO-loaded microspheres has slightly lower initial release amount in comparison to gentamicin microspheres
  - compressed microspheres showed slower release rate as compared to powdered
    - possibly due to higher surface area for powdered microspheres.
  - although powdered microspheres has better release profile, compressed microspheres are preferable for *in vivo* evaluations due to "wash-out" factor.

Conclusions

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- PLGA microspheres loaded with gentamicin and NSO are believed to have a promising potential to be developed as an alternative treatment for osteomyelitis.
- The sustained-release of both gentamicin and NSO had been demonstrated for up to 4 weeks.
- *In-vivo* data showed promising safety and efficacy effects.


The End