In vitro and In vivo release of Gentamicin from biodegradable implantable discs

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INTRODUCTION
Osteomyelitis is an infection of the bone and successful treatment involves the thoroughly debridement of the affected bone and the tissue by a surgical procedure and implantation of a composition of antibiotics that gives sustained release of the drug for a period of about 6-8 weeks. The current treatment is done by poly methyl methacrylate (PMMA) beads loaded with Gentamicin and PMMA being non-degradable and will become a foreign body after 14 days. The current study aims to develop a biodegradable composition that gives sustained release and hence reducing the necessity for a second surgery. Gentamicin loaded discs were produced by compressing microparticulate-Gentamicin mixture obtained by spray drying a mixture of Gentamicin in a solution of a biodegradable polymer Theoretical drug loading up to 25% were studied and it was observed that 10% drug loading was optimum for Gentamicin to be used as solid in spray drying. The results showed that about 60 percent of the drug is released in about 5-6 days and the remaining drug is released in about 30 days in total. In vivo study was carried on rabbit femur and the local area and systemic concentration of Gentamicin was monitored.

MATERIALS AND METHODS
One gram of the required polymer is dissolved in 50 ml of ethyl acetate (if two types of polymers are used, then the total weight of polymer is one gram) and Gentamicin is mixed with the solution. The mixture is ultra sonicated at 15 W for three times, for 20 seconds each time (Misonix Inc. XL 2000, USA). The mixture is then spray dried in Buchi 191 Mini Spray Drier (Flawil, Switzerland) at 70°C, airflow rate of 700NI/h; pump feed rate of 30 % and an aspirator ratio of 100 %. The outlet temperature was observed to be between 45 and 50°C. The microparticles obtained were freeze-dried (Christ Alpha 1-2 Model 10020G, Germany) over night for the elimination of the solvents that may be present. For the preparation of discs the microparticles were weighed and the discs were prepared in 25mm evacuable die at 2 ton pressure for 5 minutes using a Graseby Specac (Orpington, Kent, United Kingdom) set up. The discs thus prepared were again stored in dessicator. Scanning electron microscope is used to study the morphology of the micro particles. A Joel JFC-1100E, Japan instrument was used. The particle size distribution was studied by laser light scattering technique using a Brookhaven 90 Plus particle size analyzer, Holtsville, New York, USA. [11]

In vitro release and In vivo study
In vitro release from the discs was studied by placing them in 20 ml PBS solution pH 7.4 and 37°C in a water shaker bath at 110 rev/min. At specified intervals of time the supernatant solution is taken out and is replaced with fresh PBS solution [14-20]. White New Zealand rabbits weighing between 2.2 and 2.8 kg are used in vivo study. Anesthesia was given at 35 mg/kg of Ketamine and 5 mg/kg of Xylazine. During the surgical procedure, a hole of about 5 mm * 10 mm is drilled in the femur bone’s supercondylar region [21-23]. The medullar cavity is reached while avoiding damage to the ligament insertions and the PMMA spacers are implanted in the bone. A continuous suture closes the surgical wound and the rabbits are left for free movement in the cages after recovery from anesthesia. 2 weeks later, the same procedure was done and replaced the spacer with the polymeric discs containing Gentamicin.

RESULTS AND DISCUSSION
In vitro release
Gentamicin is a hydrophilic drug and comes out of the composition very fast. It was observed that all of the Gentamicin was released from the microspheres in less than a day (results not shown). It may be possible due to the fact that the microparticles were very small of the order of 1-2 μm and that Gentamicin is very hydrophilic. It was observed that about 5 % of Gentamicin in the initial few hours of in vitro release which is possibly due to the release from the surface of the disc. This was followed by a lag phase, a period that is dependent on the degradation of the PLGA used. This was followed by a second release, which lasted up to the period where the disc degrades completely (fig 1).

In vivo release
In vivo release from the discs was almost the same as in vitro study. The first day reached the peak release concentration and the days after it maintained the sustain release for 28 days above therapeutic level. (Fig 2)

CONCLUSION
According to our animal study in first batch, the condition looks like the routine debridement for fresh open fracture with severe contamination, we could implant our product to prevent infection as Septopal® (PMMA with Gentamicin). And it has advantage that we do not need to remove the foreign body again in following period as PMMA beads. In chronic osteomyelitis condition, after thoroughly debridement, our product could act as space filler and constant release the antibiotics for treating chronic infection according to our second batch of animal study.

In conclusion, this is a good idea of product for treating osteomyelitis as Septopal® and it dissolve in a few days by itself. And in the future, the growth factor may be added for better bony union.

REFERENCES

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