1. TITLE OF THE PROPOSED WORK:

Formulation and Development of Biodegradable implant for Treatment of bone infections

2. INTRODUCTION

A major development in recent years has been the fabrication of implantable drug delivery system using biocompatible and biodegradable polymers [1, 2]. Polymeric compounds have been widely used in designing the implantable controlled release delivery systems due to the various advantages they offer such as predictability of drug release profile and ease of fabrication [3].

Implants are generally administered by injecting subcutaneously by minor surgery or by the placement of a surgeon directly to or near the site required. In the case of non-biodegradable polymers, an implanted dosage form has to be removed at the end of the release period while the use of biodegradable polymers avoid a second surgical intervention.

In orthopedics, biodegradable polymers are frequently used to produce fixation rods, plates, screws, suture anchors and sutures [4]. Investigational uses of these materials include augmenting repair of musculoskeletal tissues, either as scaffolds for tissue in growth or carriers of tissues, cells or bioactive agents. Ongoing research in this field strongly suggest that these materials will also be used more extensively as fillers for bone defects and scaffolds for cartilage and meniscal repair.

Several antibiotic implantable drug delivery systems have been developed for treatment of bone infection. The advantage of this approach is that an effective drug concentration is attained at the site of infection, while the systemic drug concentration remains very low [5]. The main disadvantage is that the implants should be removed at the end of the treatment period. Implantable biodegradable polymer systems have a unique advantage in that the dosage form need not be removed from the body and thus saves cost and risk for the patient.
The use of fluoroquinolones for the treatment of osteomyelitis is now an established systemic therapeutic approach [6]. Materials like tricalcium phosphate and hydroxyapatite as fluoroquinolone carriers for treating bone infection have been studied [7]. In addition, biodegradable polymers such as poly(lactic acid), polyurethanes [8] and cross-linked high amylose starch [9,10] have been tested and show potential effectiveness as quinolone carriers in local treatment of bone infection.

For local treatment, as well as prophylaxis of bone infections, nonbiodegradable polymethylmethacrylate (PMMA) bone cement or bead implants impregnated gentamicin have been used for decades [11]. The release of gentamicin from PMMA beads is slow and incomplete [12]. As PMMA implants are not degradable, surgical removal of these implants is recommended (or necessary) when the drug is depleted. Furthermore, experimental data suggest that slime or glycocalyx-producing bacteria may adhere to the PMMA, even when this still contains antibiotic to which the bacteria are sensitive [13]. Pharmacokinetic studies of gentamicin-impregnated acrylic cement in 10 patients undergoing total hip joint arthroplasties indicated that only 5.78% of the total quantity implanted was released over 15 days [14]. These disadvantages have generated interest in developing an absorbable carrying material for depot administration of antibiotics.

Biodegradable polymeric carriers, on the other hand, obviate the need for surgical removal of these carriers or implants at the end of therapy or when the drug is depleted. Polylactide and its copolymers have attracted much attention as materials for drug delivery [15,16] reported the use of encapsulated microspheres of antibiotics in the prevention of infection in a rabbit open tibia fracture model. They found high local levels and low systemic levels of antibiotics, and low incidence of infection in rabbits treated with antibiotic encapsulated microspheres. Sampath et al. [12] intended to fabricate poly(l-lactic acid) (PLA) implants containing gentamicin sulphate for the treatment of osteomyelitis by prolonged localized delivery of drug. In their in vitro release test, cylindrical poly(l-lactic
acid) implants obtained by compression of microcapsules, ranging from 178±444 mm, released an amount exceeding 80% gentamicin sulphate within 3 weeks. In contract, Miyamoto et al. [17] pointed out that poly(dl-lactic acid) homopolymers with high molecular weights of 105 000, 21 000 and 3300 were inappropriate as a bone morphogenetic protein (BMP) carrier, primarily because they produced strong foreign-body reactions or chronic inflammation, and were too slowly absorbed to be replaced by induced bone.

Teicoplanin is a new glycopeptides antibiotic providing both in vitro and clinical efficiency against most gram positive organisms including Staphylococcus aureus, Staphylococcus epidermidis and Cornyeobacterium ssp in patients with a variety of moderate and severe infections especially osteomyelitis. Teicoplanin does not seem to be ototoxic and appears less nephrotoxic and is well tolerated in humans and animals [18, 19, and 20].

Gatifloxacin is an extended-spectrum fluoroquinolone with improved Gram-positive and anaerobe coverage compared with older agents such as ciprofloxacin. The methoxy substituent at its C-8 position ensures a greater inhibitory action on DNA gyrase and topoisomerase IV of Gram-positive bacteria and prevents the emergence of mutant strains in Staphylococcus aureus [21]. Gatifloxacin has been used for a variety of clinical conditions including genitourinary infections [22], lower respiratory tract infections [23], community acquired pneumonia [24], acute bacterial exacerbations of chronic bronchitis [25], acute bacterial rhinosinusitis [26], and recurrent acute otitis media [27].

Polycaprolactone (PCL) is a biodegradable polyester with a low melting point of around 60°C and a glass transition temperature of about –60°C. PCL is degraded by hydrolysis of its ester linkages in physiological conditions (such as in the human body) and has therefore received a great deal of attention for use as an implantable biomaterial. In particular it is especially interesting for the preparation of long term implantable devices, owing to its degradation which is even slower than that of polylactide.
Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It has a number of commercial and possible biomedical uses. Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.) and cell walls of fungi.

Alginate is naturally derived linear polysaccharide composed of (1-4)-linked D-mannuronic acid (M units) and α-L-guluronic acid (G units) which vary in proportion and sequential distribution along the polymer chains [28]. Alginate is considered as biocompatible and forms spherical beads in the presence of divalent cations like Ca$^{2+}$ and Ba$^{2+}$ through ionic gelation [29]. Calcium cross-linked beads have been used in many biomedical applications [30].
3. MAJOR OBJECTIVES OF THE PROPOSED STUDY

The proposed study has been undertaken with the following major objectives:

1. To synthesis biodegradable implants from biocompatible polymers such as polycaprolactone, chitosan, poly(lactide-co-glycoside) and sodium alginate.

2. To encapsulate antibiotic drugs like gentamicin, gatifloxacin, ciprofloxacin, teicoplanin into these polymers to yield drug-loaded polymeric implants.

3. To carry out detailed investigation of in vitro and in vivo release of drugs from these implants under physiological conditions under different experimental parameters.

4. METHODOLOGY

The proposed study shall be carried out in following three phases.

PHASE I: Preparation of polymeric implants

(i) Preparation of drug-loaded polycaprolactone implant

Definite amount of polycaprolactone shall be dissolved in dichlorometane and after its complete dissolution, pre-weighed quantity of antibiotic drugs shall be mixed under high agitation speed to get uniform
dispersion or solution as the case may be. The dispersion shall be cast onto Teflon plate and dried overnite at 5°C. Finally, the implant shall be taken out and sterilized by \( \gamma \)-radiation.

(ii) **Chitosan based implant**

Drug – loaded chitosan implant shall also be prepared in almost similar way. The difference in method of preparation is that known amount of chitosan shall be dissolved in 2% lactic acid or acetic acid and then a pre-weighed quantity of drug shall be added under high agitation speed. Then the dispersion/solution so formed shall be placed on Teflon plate and kept at 50°C overnite. Finally, the films shall be peeled off and kept in air-tight containers for further use.

(iii) **Sodium alginate based implant**

The sodium alginate based implants shall be prepared by dissolving pre-weighed quantity of drug in 3% aqueous alginate solution under constant stirring till a uniform dispersion is obtained. The solution will be poured into Petriplate and will be dried at 60°C. The films thus obtained shall be crosslinked with \( \text{CaCl}_2 \) solution via ionotropic gelation[31].

**PHASE II : Characterization of Implants**

(i) **Differential Scanning Calorimetry (DSC)**

Samples of pure drug, polymer, and prepared implant were heated over the range of 25°C to 300°C with a heating rate 10°C/min.

(ii) **Tensile Strength**

Rectangular strips of 20 × 15 mm size were cut and strained to break at a constant crosshead speed of 20 mm/min using Instron 5842 (Instron, Canton, MA, USA) using the associated software “Merlin.” Tensile strength at breakpoint will be as measured.
(iii) **XRD-studies**

The XRD apparatus (Philips PW 1820) powder diffractometer, shall be used to investigate the crystallinity and phase content of polymeric film. The diffraction data will be collected from 2 to 60°, 2°θ values with a step size of 0.02° and counting time of 2 s·step–1 at λ i.e. 1.54 Å.

(iv) **Microscopy study**

An approximate idea of the morphology of polymeric films will be deduced by using Optical microscope, MIOTIC DIGITAL MICROSCOPE DMWB-series. In order to study the morphology of the prepared composites SEM will be as carried out on STEREOSCAN, 430, Leica SEM, IICT, Hyderabad.

**PHASE III** :

(i) **Minimum Inhibitory Concentration (MIC)**

MIC will be determined for ATCC 33591 by the agar dilution method as described in the Clinical and Laboratory Standards Institute (CLSI) recommended methods using Mueller-Hinton agar and direct colony suspension, equivalent to a 0.5 McFarland 130 standard and incubated at 35°C for 20 hr. Dilutions of GAT shall be started at 512 µg/L. The MIC will be considered to be the lowest concentration of GAT that prevented visible growth (National Committee for Clinical Laboratory Standards 2003). The MIC shall be confirmed by the E-test (AB Biodisk, Solna, Sweden) performed according to the manufacturer’s instructions to expand the range of dilutions available.

(ii) **Sterility Test**

Sterility tests shall be performed for the sterilized membrane to assure sterility. Three samples of GAT implants sterilized by gamma radiation shall be each placed in 20 ml vials of tryptic soy broth and incubated at
37°C for 3 weeks. Subcultures will be made onto blood agar after the 3 weeks for detection of any growth.

(iii) **In Vitro Drug Release**

Three weighed and sterilized pieces of antibiotic implants shall be placed each in 2 ml of PBS (pH 5.3) and incubated at 37°C for 24 hr. A predetermined amount of drug content shall be used to maintain the sink condition. The implant will be removed, shaken free of excess PBS, transferred to fresh 2 ml aliquots of PBS after 2 hr, 4 hr, then every 24 hr for 2 weeks then every week for 2 more weeks. Samples of removed PBS will be stored at –70°C until a microbiological disc diffusion assay shall be performed.

Disc diffusion assays shall be performed to determine the antibiotic concentration in the samples. An aliquot of 0.1 ml of overnight culture of *Bacillus subtilis* ATCC 6633 suspension will be added per 100 ml of antibiotic agar medium one (Difco, Detroit, MI, USA). First, 5 ml of this seeded agar shall be aseptically pipetted into Petri dishes. Standard 2-fold serial dilutions of GAT shall be made in PBS (pH 5.3), producing standard concentrations ranging from 0.002 to 5 mg/ml. Second, 20 µl of each in vitro release sample and standard concentration will be added to each of three sterile blank 6 mm filter paper discs placed on the seeded plates. The plates shall be incubated overnight at 37°C. The diameter of the zone of inhibition for each standard and in vitro release sample will be measured. The unknown concentrations for the in vitro release samples will be determined by comparing their respective zone size means to the standards [32]

(iv) **In Vitro Inhibition of MRSA**

Test tubes containing Mueller-Hinton broth (Oxoid Ltd., 170 London, UK) inoculated with 5 × 10⁶ CFU of MRSA and GAT implant (equivalent to 40 mg drug/10 ml broth) shall be incubated at 37°C
together with three control tubes without implants. The implants shall be washed and switched to a new set of inoculated tubes each day, and tubes will be sampled for colony counts to determine bactericidal effect.

(v) **In Vivo Release**

Rabbits shall be anesthetized using an intramuscular injection of 45 mg/kg ketamine (Tekam, Hikma Pharmaceuticals, Amman, Jordan) and 8 mg/kg xylazine (Rompun, Bayer, Germany). The left hind leg be shaved and disinfected. An incision of 2 cm will be made on the medial side of the left leg just below the knee. Fascia and muscles shall be dissected to expose the tibia. A saw will be used to make a 0.5 × 1 cm opening in the bone and the implant will be inserted in a dose of 40 mg/kg then the site sutured. Three rabbits will be sacrificed at 1, 2, 3, and 4 weeks after implant insertion. The bones shall be extracted and the drug assayed in the bones.

(vi) **Drug Assay in Bone**

Bones will be prepared for assay by dissecting them free of all soft tissues and the implant removed. Tibial diaphysis will be broken down into small pieces, crushed, weighed and suspended in PBS (pH 5.3). Then 1ml of buffer will be used per 0.5 g bone. The crushed bone was shaken on a vortex mixer for 5min. Antibiotic in the supernatant fluid will be assayed by the described disc diffusion method. Drug concentrations will be determined from semilog standard curves [33].

5. **SIGNIFICANCE OF THE STUDY**

In the proposed study biodegradable implants, loaded with suitable antibiotic drugs, are to be synthesized and investigated for their in vivo efficacy against bone infection. If the results are encouraging and positive, then there are chances of using these biodegradable implants for treatment of bone infections and may replace synthetic non-degradable polymeric implants.
REFERENCES


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