Biodegradable PLGA barrier films for the localized release of bone regenerative bis-phosphonate drugs for periodontal disease

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**Introduction:** Periodontal disease is characterized by gingival inflammation leading to bone loss in the jaw and a loosening of teeth. Current surgical interventions involve deep exposure of the gum, debridement of necrotic tissue and suturing of the gum. Unfortunately, epithelial cells quickly invade the debrided space, inhibiting bone regrowth around the tooth. Surgeons attempt to inhibit epithelial invasion by applying non-degradable polymeric barrier films around the tooth which require a secondary operation to remove the film when the area has healed. We have recently described biodegradable polymeric films made from poly (lactide co-glycolide) (PLGA), made elastic by the addition of excipients, suitable for periodontal barrier applications, that do not require surgical removal. (Owen et al 2005) These films were grooved to promote the directional growth of osteoblasts and facilitate rapid bone replacement in the periodontal space (guided tissue regeneration). Recent studies have shown that local delivery of bisphosphonates (such as alendronate) can improve bone growth around dental and orthopedic implants (Yoshinari et al. 2002, Astrand and Aspenberg 2004). Most of these studies have applied the bisphosphonate either topically into the implant cavity or as a drug coating to the implant itself, but neither of these approaches affords controlled drug release necessary for long term drug effects. The objective of this study was to encapsulate drugs in PLGA periodontal barrier films, that might be released in a controlled manner to inhibit bacterial growth (antibiotic: tetracycline) and to promote bone growth (osteoclast inhibitor/osteoblast promoter: alendronate).

**Materials and Methods:** Film manufacture: Teflon squares measuring 1 cm by 1 cm were applied to glass slides as film templates. Film casting solutions were prepared by dissolving PLGA (85/15 ratio) containing the water soluble excipients PEG 600 or diblock copolymer (and ground alendronate or tetracycline if applicable) in a small quantity of dichloromethane (DCM) to a total final concentration of 10% w/v. The mixtures were vigorously mixed by vortexing and ultrasonication until homogeneous. An aliquot was cast over the template. The glass slides were placed in a loosely closed plastic container and placed in the refrigerator for 3-5 days to let the solvent evaporate slowly.

Film characterization: Excipient miscibility with PLGA was measured using differential scanning calorimetry (DSC). PLGA degradation and excipient release were measured using gel permeation chromatography. Drug release was measured following incubation of films (containing different concentrations of drugs or excipients) in PBS, pH 7.4, at 37oC, using HPLC drug analysis methods. The effect of alendronate, or drug released from films, on osteoblast function was measured by counting following sulforhodamine B/ Hoescht staining or by quantitative microplate MTS assay. Long term effects on osteoblast function (4 week incubation) were measured using established assays for alkaline phosphatase (nodule staining and counting) and mineralization (alizarin red staining).

**Results:** DSC analysis of films established the miscibility of the polymeric components. More than 80% of the excipients were found to release over a period of 4 weeks from the films (GPC analysis). Tetracycline and alendronate released from all films with a burst phase over 5 days followed by a slower more sustained release over the following weeks. The inclusion of PEG 600 or diblock copolymer allowed for a concentration dependent increase in release rates for both drugs. Alendronate at concentrations greater than 0.25% (w/v) inhibited osteoblast proliferation and were not used in further cell studies. MTS assays of cell viability (proportionally related to cell number) showed no effect of alendronate on osteoblasts. However, cell staining and counting methods established that 0.25% concentrations of drug caused an increase in cell proliferation rates at 1,2 and 4 days. Osteoblasts grew well on surface activated PLGA films (glow discharged). At 4 weeks there was no significant difference in ALP or mineralization activity between cells grown on 1.25% alendronate loaded or control PLGA films. Studies that involve longer incubation times and effects on osteoclast-like cells are in progress.

**Discussion:** Both drugs encapsulated and released well from these films. The release rates fit the surgical healing timelines, with the release of therapeutically relevant quantities of both drugs over a period of more than one month. PLGA films degrade in vivo over a 2-6 month period. The films are initially elastic but swell and stiffen in aqueous media, suiting the surgical site where edge-sealing around the tooth and the maintenance of a void shape for bone infiltration is preferred. Overall, these drug-loaded films may offer a prolonged anti-infective and bone-regenerative drug delivery system whilst simultaneously providing a biodegradable barrier for preferred tissue regeneration.

