Unique Biocompatible Dipeptide-Based Biodegradable Polymeric Blends for Musculoskeletal Regeneration: Poly[(glycine ethyl glycinato)(phenyl phenoxy)phosphazene] – Poly(lactide-*co*-glycolide) *In vitro* and *In vivo* Degradation and Biocompatibility Studies

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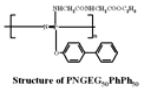
Statement of Purpose: Tissue engineering has emerged as an alternative strategy to regenerate or replace damaged or lost tissues. In this approach, biodegradable polymers play a paramount role as transient substrates that can be replaced by the newly formed tissues *in vivo*. Due to the limitations associated with the current polymers such as the acidic degradation from poly(lactide-*co*-glycolide)(PLAGA), there is a great need for the development of new biomaterials with controllable degradation, non-toxic and neutral degradation products, appropriate mechanical properties, and good biocompatibility. The "self-neutralizing" ability and controlled degradation of the PLAGA-polyphosphazene blends have been demonstrated by blending PLAGA with glycine ethyl ester co-substituted polyphosphazenes (EG-PPHOS)[1]. The objective of the present study was to develop novel biocompatible materials by blending PLAGA with glycine dipeptide-based poly[(50%glycine ethyl glycinato)(50%phenyl phenoxy)phosphazene] (PNGEG/PhPh) since dipeptide can provide multiple hydrogen bonding sites to achieve complete miscibility with PLAGA[2], while phenyl phenoxy group possesses good biocompatibility and high mechanical strength[3,4].



Structure of PNGEG$_{50}$PhPh$_{50}$

Methods: PLAGA 50:50 ($\overline{Mw}$ 34 kDa) was purchased from Boehringer Ingelheim KG. PNGEG/PhPh was synthesized via a two-step polymerization route[2]. BLEND25 and BLEND50 having a PNGEG/PhPh:PLAGA weight ratio of 25:75 and 50:50 were prepared[3]. *In vitro* degradation study was conducted in phosphate buffered saline (PBS)[1]. At specific time points (1, 4, 7, 10, 12 weeks), the matrices were removed from the media and dried under vacuum to constant weight. The results were reported as percent mass loss versus time. The media were also recorded by pH meter. The molecular weight of the degraded matrices was determined using GPC. Samples were visualized by SEM. Subcutaneous implantation in Sprague-Dawley rats was used to evaluate the *in vivo* degradation and biocompatibility of the blends. Animals were sacrificed at predetermined time points (2, 4, 7, 10, 12 weeks). The thickness of the inflammatory zone (H&E) and collagen deposition (MTS) for each implant was expressed as the average value of at least three readings per slide of six slides at each time point. The biocompatibility of the materials was classified as Level 1, Level 2, Level 3, or Level 4[4].

Results/Discussion: The blends showed complete miscibility, higher mechanical properties, and better *in vitro* osteocompatibility as compared to PLAGA[2]. Fig. 1a confirms that the degradation products of PPHOS were able to neutralize the acidic degradation products of PLAGA and lead to a significant increase in the pH of the degradation media. Also as shown in Fig. 1b, , both the blends degraded at a slower rate than PLAGA with a total percent mass loss of 82% and 69% for BLEND25 and BLEND50 after 12 weeks of degradation, respectively. It suggests the degradation of blends can be effectively controlled by varying the blend composition. Further molecular weight measurements indicated the two polymer components in the blend degraded in an interactive pattern. Most interestingly, as seen from Fig. 2, representative SEM micrographs of blends after degradation showed unique 3D interconnected porous structures with suitable pore size (>10μm) for cell migration and tissue in-growth. These unique changes in the polymer morphology after degradation can be beneficial for osteoconduction and tissue in-growth, which makes such blend materials potential scaffold candidates for accommodating tissue regeneration. Furthermore, *in vivo* biodegradation and biocompatibility evaluation of PPHOS-PLAGA blends using rat subcutaneous implantation demonstrated a similar degradation mechanism and showed Level 1 biocompatibility. Fig. 3 shows both the blends induced less inflammatory responses and thinner fibrous capsules than PLAGA. As indicated by the arrows in Fig. 4, the porous structures formed during blend degradation showed great potential in directing and hosting tissue in-growth *in vivo*.

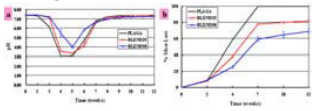


Fig. 1. degradation profiles of blends over 12 weeks: (a) pH of the media, (b) percent mass loss of blends and PLAGA.

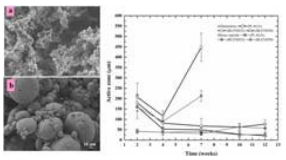


Fig. 2. (left) SEM micrographs of unique 3D porous structures after 12 weeks of degradation: (a) BLEND25, (b) BLEND50; **Fig. 3. (right)** Change of thickness of the immune response zone with time for PLAGA and blends.

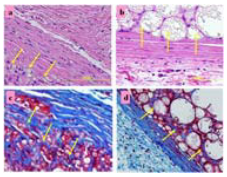


Fig. 4. Comparisons of lumen wall characteristics (a, b, H&E, 40x) and (c, d, MTS, 10 x)) after 84 days of implantation. (a, c): BLEND25, (b, d): BLEND50. Arrows indicate the polymer bead formation. Most interestingly, tissues readily grew into the pores while the vascularized collagen formation occurred surrounding the polymer beads.

Conclusions: We have successfully demonstrated that the biocompatible dipeptide-based polyphosphazene-PLAGA blends are very promising biodegradable materials for musculoskeletal regeneration. The unique degradation of these biocompatible materials offers a new paradigm in scaffold-based tissue regeneration.

References: 1. Laurencin et al., Biomaterials 23(7);1667 (2002) 2. Deng et al., 2008 WBC [2383] 3. Deng et al., Biomaterials 29(3);337 (2008); 4. Laurencin et al., J Biomed Mater Res A 77(4); 679 (2006)

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