CHAPTER IX
CONCLUSIONS AND RECOMMENDATIONS

In this research work, electrospun fibrous substrates of poly(ε-caprolactone) (PCL)/poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) without and with the presence of hydroxyapatite (HAp) or protein-loaded hydroxyapatite (HAp/protein) nanoparticles were successfully fabricated from 10 wt% of 50/50 w/w PCL/PHBV in 80/20 v/v chloroform/*N,N*-dimethylformamide solution. The blend composition of 50/50 w/w between PCL and PHBV at 10 wt% was chosen because the fibers obtained from this condition were very uniform and smooth, which appeared to support the attachment, proliferation, and differentiation of bone cells particularly well. Ovalbumin (OVA), type-I collagen (COL), fibronectin (FN), and crude bone protein (CBP) were used as model proteins.

HAp and OVA-loaded HAp (HAp/OVA) nanoparticles were synthesized by co-precipitation method using both chemical starting material (i.e., $CaCO_3$ and $CaHPO_4{\cdot}2H_2O$) and natural starting material (i.e., CaO from discarded egg shells and H_3PO_4) at various pH conditions with different amounts of OVA initially added. From the release characteristics of OVA, the HAp/OVA nanoparticle synthesized at pH 7 from the chemical starting material with around 26% of initial amount of OVA added not only had the average molar Ca/P ratio close to that of the ideal HAp in human bone but also provided the optimal amount of OVA released. Therefore, this condition was selected for preparing HAp loaded with other proteins (i.e., COL, FN, and CBP).

The potential use of the PCL/PHBV fibrous substrates filled with protein (COL, FN, and CBP)-loaded HAp nanoparticles as bone-scaffolding materials was evaluated *in vitro* in terms of the attachment, proliferation, differentiation, and mineralization with mouse calvaria-derived pre-osteoblastic cells (MC3T3-E1). Due to the highest amount of CBP released, the fibrous substrate prepared from PCL/PHBV-HAp/CBP suspension appeared to be the best among the examined fibrous substrates for supporting the growth of MC3T3-E1.

In another biomedical application, PCL/PHBV fibrous substrates with different blend ratios of 75/25, 50/50, and 25/75 w/w between PCL and PHBV containing doxycycline hyclate (DOXY) were studied for being used as drug carriers.

The cumulative amount of DOXY released from DOXY-PCL/PHBV 50/50 fibrous substrate was the greatest. This result confirmed that the blend composition of 50/50 w/w between PCL and PHBV was the most suitable for being used in controlled release system. Also, the potential use of the DOXY-loaded PCL/PHBV fibrous substrates as wound dressings was assessed by antibacterial activity against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*. The results indicated that the DOXY-loaded PCL/PHBV fibrous substrates were effective against the two pathogens, hence proving a potential application for use as both a drug delivery and a wound dressing agent.

The recommendation of the future work will be based on *in vivo* evaluation of the PCL/PHBV fibrous substrates filled with CBP-loaded HAp nanoparticles and those both filled with CBP-loaded HAp nanoparticles and simultaneously dipped into CBP solution for bone regeneration. Furthermore, the comparative study of the PCL/PHBV filled with CBP-loaded HAp in the forms of fibrous and sponge scaffolds for bone cell culture and biodegradation should be investigated.