Introduction:

Autografts and allografts have been used as the bone grafts to augment bone healing in the 18th century. However, there are concerns of donor site morbidity and transmission of disease. Therefore, the use of synthetic grafting material is emerged to regenerate diseased or damaged bone tissue. Ceramic and polymeric materials are the most commonly used synthetic materials. However, due to their poor mechanical properties, composite materials were developed instead. An ideal composite should possess good biodegradability, biocompatibility, injectability, osteogenic differentiation properties and mechanical properties that close to human bone. Poly-caprolactone (PCL) is one of the suitable candidates to be used to form composite material since it has slow degradation rate and relatively low melting point when compared with other polymers. Therefore, it allows sufficient time for bone healing and easy shaping for the scaffolds. However, the low mechanical strength and intrinsic hydrophobic properties of polymers may inhibit its use. Hence, our group has recently fabricated a biodegradable polymeric-metallic hybrid made of PCL and magnesium (Mg) to solve the problems. Mg is chosen as the incorporating material since it possesses higher mechanical properties when compared with polymers. However, in order to reduce rapid corrosion and further enhance the mechanical properties of Mg, a silane coupling agent (TMSPM) surface treatment was used to coat on the Mg granules prior hybrid fabrication. This study aims to investigate the mechanical, in-vitro and in-vivo properties of the newly developed hybrid.

Methods:

(a) The PCL-Mg hybrids were prepared by incorporating 9% Mg granules with 2 different particle sizes (i.e. 45µm and 150µm), respectively by thermal method.

(b) Compression test was conducted to study the mechanical properties of the hybrids with the use of Material Testing System (MTS).

(c) For the in-vitro studies, MC3T3-E1 pre-osteoblasts were used. MTT assay was conducted to test the cytotoxicity of the PCL-Mg hybrids. In addition, the osteogenic differentiation properties of the hybrids were checked by the ALP assay.

(d) For the in-vivo studies, two of each of the pure PCL and PCL-Mg hybrids were implanted into six 2 month old Sprague-Dawley rats, respectively. The animals were monitored and examined by micro-computed tomography at week 1 to week 4 and week 8 of post-operation.

Results:

Figure 1 shows the compressive moduli of pure PCL and PCL-Mg hybrids. The PCL-Mg hybrids have higher compressive moduli than pure PCL. The compressive moduli were significantly increased from 249MPa of pure PCL to approximately 344MPa and 331MPa of the hybrids with 9% Mg granules incorporated. No significant differences in cell viabilities were found on the pure PCL and PCL-Mg hybrids (Figure 2) from the result of MTT assay. In addition, significantly higher specific ALP activities were found on the PCL-Mg hybrids as compared to pure PCL on both Day 7 and Day 14 (Figure 3). For the animal studies, Figure 4 shows the micro-ct images and 3D models of the PCL and PCL-Mg hybrids after 2 months of post-operation. More newly formed bone was found around the PCL-Mg hybrids as compared to the pure PCL.

Discussion:

Apart from the higher mechanical properties of Mg, it is also an essential mineral to cells. In our previous in-vitro studies, a certain amount of Mg ions release (i.e. 50ppm) would help activate the osteoblastic activity so as to stimulate bone formation and enhance bone healing. However, Mg alone is not suitable to use as the bone grafting material. Hence, PCL is used to form the polymer matrix so as to make the hybrid injectable as well.

In the compression test, there were at least 25% increased in compressive moduli of the PCL-Mg hybrids as compared to pure PCL, which suggested that the incorporation of Mg granules was able to alter the mechanical properties of PCL and the values fall within to that of cancellous bone (0.3-2.1GPa). Therefore, it was expected that different mechanical properties could also be obtained with the addition of different amount of Mg granules. However, no significant difference of compressive modulus was found for different Mg particle size, this suggested that the particle dispersion with only 9% Mg granules incorporation was good enough so that the Mg granules can be evenly distributed within the polymer matrix. In addition, similar cell viabilities were found between pure PCL and PCL-Mg hybrids, which showed that no toxic effect could be found with the incorporation of 9% Mg granules. Moreover, the PCL-Mg hybrids favored osteogenic differentiation since significantly higher specific ALP activities were found on the PCL-Mg hybrids as compared to pure PCL on days 7 and 14. The specific ALP activity can also demonstrate the results that obtained in in-vivo studies. Hence, more newly formed bone can be found around the PCL-Mg hybrids as compared to the pure PCL.

In summary, we report here that with the incorporation of Mg into polymer, a novel polymeric-metallic hybrid, which is biodegradable, injectable, bioactive and with suitable mechanical properties comparable to human bone can be fabricated. However, further studies include the mechanical test during degradation is still needed.

References:

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Figure 1. Compressive moduli of pure PCL and PCL-Mg hybrids. The addition of Mg is able to enhance the compressive moduli of pure PCL. (*p<0.05)

Figure 2. Cell viabilities of pure PCL and PCL-Mg hybrids. No toxic effect was found as the hybrids have similar cell viabilities as pure PCL.

Figure 3. Specific ALP activities of pure PCL and PCL-Mg hybrids. Significantly higher activities were found on the hybrids on days 7 and 14 than pure PCL. (*p<0.05)

Figure 4. Micro-ct images and 3D models of the newly formed bone (red arrows) around the implants (yellow arrows). More new bone was found around the PCL-Mg hybrids as compared to pure PCL.