POROUS CHITSAN-GELATIN SCAFFOLDS EMBEDDED WITH PLGA NANOPARTICLES FOR BONE REPAIR.

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Introduction

In the domain of tissue engineering, the repair of skeletal defects is an immediate clinical need as impaired fracture healing or non-union bone defects often result in a functional disability for which there is no effective therapy [1].Conventional tissue engineering strategies utilize combination of biodegradable porous scaffolds and bioactive molecules such as growth factors to mimic natural processes of healing [2]. However, such approaches often produce unpredictable results, probably due to the short biological half life of bioactive molecules and lack of long term stability and tissue specificity [3]. The aim of this work is to prepare chitosan-gelatin (CH/G) scaffolds embedded with PLGA nanoparticles for the purpose of localized growth factor delivery at a pre-determined rate and to improve the therapeutic efficiency of scaffolds by mimicking the endogenous release of bioactive substance(s).

Materials and Methods

Chitosan (CH) and Type A gelatin (G) from porcine skin were supplied from Sigma-Aldrich.

Figure 1. Fabrication of porous CH-G scaffolds. (Ref. 4)

Figure 2. Schematic representation of formation of PLGA nanoparticles (Ref. 5)

Nanoparticles-embedded scaffolds were constructed by dispersing various amounts of PLGA nanoparticles (PLGA NP) into 1 ml of CH/G blend solution before the pre-frozen and freeze-drying steps, which were described above. Scaffolds were characterised for their stability (swelling and dissolution tests), morphology (scanning electron microscopy, SEM).

Conclusions

Scaffolds incorporated with PLGA nanoparticles displayed lower swelling than CH/G scaffolds without nanoparticles. Incorporation of the hydrophobic PLGA nanoparticles in the relatively hydrophilic CH/G scaffold modulated the porous structure and tensile strength of the scaffold. Studies are in progress to evaluate the release kinetics of particles embedded into CH/G scaffolds. GP crosslinked CH/G blends embedded with PLGA nanoparticles containing suitable growth factor can be used in the form of porous scaffolds, to enhance bone regeneration.

References

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Results and discussion

The use of CH/G blends as scaffolding materials may affect cell adhesion, scaffold bioactivity, tissue remodelling process and the quality of the regenerated tissue. Genipin-crosslinking stabilized the chitosan-gelatin blends in water solution and pre-freezing at -20°C warranted the uniform gel formation. Freezing resulted in the formation of ice crystals inside polymer solution which act as porogens during freeze drying process that resulted in a porous (160-200 microns) 3D polymer scaffold (Fig.3). PLGA nanoparticles formed ere spherical in shape and displayed a smooth surface with an average size below 200 nm (Fig.4). PLGA nanoparticles were uniformly distributed and located in the walls or void spaces of the CH/G scaffold (irrespective to the amount of nanoparticles used) as witnessed in SEM image (Fig.5).

Figure 3. Porous CH-G scaffolds

Figure 4. PLGA nanoparticles.

Figure 5. Porous CH-G scaffolds embedded with PLGA nanoparticles.

Scaffolds with PLGA nanoparticles displayed heterogeneous porous structure (Fig.5) which could be attributed to the fusion of two or three adjacent pores by the rupture of common pore walls. This might be due to the steric effect of the embedded nanoparticles or to the strong water repelling force exerted by the hydrophobic nanoparticles. Furthermore, scaffolds containing PLGA nanoparticles displayed lower % swelling and higher mechanical properties than the ones without particles (Fig.6&7). Weight loss in water due to CH/G release ranged between 29 to 49 % for scaffolds after 10 days immersion in Phosphate buffered saline(PBS) (Tab. 1).

Figure 6. %Swelling of scaffolds in PBS. Reported values are the averages and bars indicate the S.D. (n=3)

Figure 8. Supernatant pH changes during the dissolution of scaffolds as a function of time at 37°C. Reported values are the averages (n=3)

Figure 7. Compressive Young's modulus for Scaffolds. Reported values are the averages and bars indicate the S.D. (n=3)

Group	Initial Wt. mg (mean±S.D.)	Final wt. mg (mean±S.D.)	% wt loss
CH-G scaffolds	250.5±12.1	127.3±13.8	49.16 ±0.63
CH-G with 5 mg/ml PLGA NP	258.4±20.6	163.7±31.8	36.6 ±6.43
CH-G with 10 mg/ml PLGA NP	235.9±16.24	126.6±28.6	46.63 ±18.43
CH-G with 20 mg/ml PLGA NP	240.1±9.1	169.2±11.1	29.50 ±7.10

Table 1. Dissolution of scaffolds after 10 days of immersion in PBS.

The measured pH of the incubation media indicated acidic conditions after immersion of the obtained scaffolds in PBS, which could be attributed to the degradation of chitosan. In case of control samples the maximum acidic condition occurred after first week then pH raised closure to initial pH. On the other hand, samples containing PLGA particles displayed more acidic conditions probably due to the degradation of PLGA particles. (Fig. 8)

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