Biomimetic nano-fibrous scaffold for multiple factor delivery

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Introduction: In natural tissue/organ regeneration, a series of events involving numerous growth factors and cytokines are orchestrated by the human body. For successful tissue regeneration, therefore, it is desirable to design a scaffold capable of delivering various biological signals in a controlled fashion.
Nano-fibrous scaffold, mimicking natural extracellular matrix at nano scale, has been demonstrated for advantageous cell-scaffold interactions (1). Biodegradable micro/nanospheres have been widely used for controlled delivery of biological factors such as hormones and growth factors (2,3). In this study, we incorporate multiple types of factor-containing nanospheres onto a biomimetic nano-fibrous scaffold to achieve individualized release profiles.

Methods: Poly(D,L-lactic acid-co-glycolic acid) (PLGA) nanospheres (NS) were prepared by a double emulsion method (2). Three PLGA copolymers (PLGA50-6.5K and PLGA50-64K: LA/GA=50/50, Mw=6.5kDa and 64kDa; PLGA75-113K: LA/GA=75/25, Mw=113kDa) were used to encapsulate three fluorescently labeled proteins: FITC-BSA (green), TRITC-BSA (red), and AF350-BSA (blue). Three-dimensional (3-D) biomimetic nano-fibrous poly(L-lactic acid) (PLLA) scaffolds were prepared as described elsewhere (4). Protein-containing NS were incorporated into prefabricated scaffolds, resulting in 0.8 mg NS per scaffold. The degradation and release kinetics were investigated for both NS alone and NS-incorporated scaffold (NS-scaffold) in PBS (10 mM, pH=7.4).

Results/Discussion: Biomimetic 3-D PLLA scaffolds with high porosity (~98%), tuneable macropores and interpore openings, as well as nano-fibrous pore wall structures were obtained. Three different fluorescent BSA-encapsulated NS (TRITC/PLGA50-6.5K; FITC/PLGA50-64K; and AF350/PLGA75-113K) were simultaneously incorporated into single biomimetic nano-fibrous scaffold, and the NS were uniformly distributed throughout the scaffold (Fig. 1).

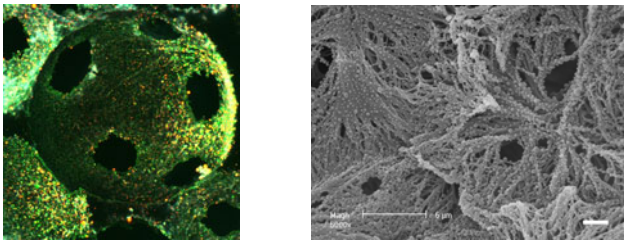

Figure 1. Laser scanning confocal microscopy (LSCM) image (left, ×200) and SEM micrograph (right, ×5000) of NS-scaffold.

Importantly, the interconnected macroporous structures and nano-fibrous pore wall features were well retained after NS incorporation. This unique characteristic would not only allow effective release of biological molecules from a scaffold, but the resulting scaffolds would also facilitate cell seeding and migration, mass transfer, and tissue organization.
The release of three labelled BSAs from single scaffold was shown in Fig. 2. Three individualized protein release profiles were obtained over a 2-week period. After initial burst release, the scaffold released TRITC-BSA from PLGA50-6.5K NS at a fast rate of about 3% per day while it released FITC-BSA and AF350-BSA from PLGA50-64K and PLGA75-113K NS at relatively slower rates of about 1% and 0.5% per day, respectively.

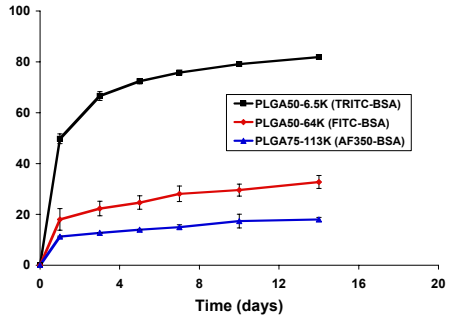

Figure 2. In vitro release of multiple fluorescently labeled BSAs from a NS-scaffold.

The degradation rates of the three NS were distinctly different because of the different molecular weights and/or LA/GA ratios of the copolymers. PLGA50-6.5K degrades faster than PLGA50-64K while PLGA75-113K degrades the slowest. The faster degradation of NS led to the faster release of the factor. It was also found that the overall release patterns were similar between NS-scaffold and the free NS. The results indicated that the release kinetics from NS-scaffold which is similar to that from NS alone, can be effectively adjusted by tailoring the degradation rates of the NS.

Conclusion: Multiple molecules released at individualized rates have been achieved. This novel NS-scaffold allows us to program biological signals into a 3-D scaffold to regulate the cellular activities and potentially afford more predictable tissue regeneration.

Acknowledgements
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References:
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