Biomimetic apatite-coated chitosan based scaffolds for bone regeneration

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Statement of Purpose: Tissue engineering substitutes suggested many alternative strategies using sophisticated biocompatible scaffolds for treatment of bone defects[1]. Chitosan (CH) is a naturally derived, biocompatible polysaccharide used for pharmaceutical and biomedical applications. Although CH has been shown to be osteoconductive and enhance bone formation, CH scaffolds alone may not possess the necessary mechanical strength for implantation. The mechanical properties of CH scaffold can be enhanced by complexing them with anionic natural polymers such as alginate, hyaluronic acid, or chondroitin sulfate[2]. The scaffolds provide not only structural support for cell growth but also provide the surface properties important for cellular responses and bio-molecular releasing. Thus, the scaffold material may influence the activity of progenitor cells and final release kinetics of growth factors. This study investigates cell-scaffold and protein-scaffold interactions with bone marrow stromal cells and model proteins with net positive (histone) and net negative charge (bovine serum albumin, BSA) from three substrates: chitosan (CH), chitosan/chondroitin sulfate (CH/CS), and chitosan/alginate (CH/AG). To better support cell growth and control release of proteins, we employed apatite as the coating of the scaffold and determined the effects of apatite coating on cell spreading, proliferation, osteogenic differentiation, and release kinetics of model proteins.

Methods: CH scaffolds were fabricated by lyophilizing 3% CH (Sigma, St Louis, MO) solution followed by crosslinking with tripolyphosphate solution. The composite scaffolds were prepared by mixing CH solution with CS (Sigma) or AG (Sigma) solution. The CH/AG scaffold was cross-linked with $CaCl_2$. For apatite coating, the obtained scaffolds were incubated in simulated body fluids as described previously[3].

The scaffolds were examined by SEM (FEI, Hillsboro, OR) for morphology, electro-mechanical testing machine (Instron, Norwood, MA) for mechanical strength, and ATR-FTIR (San Jose, CA) for chemical structure. To determine release kinetics of proteins from scaffolds, fluorescence-conjugated model proteins, BSA or Histone, were lyophilized on the scaffolds and incubated in PBS at 37°C. The fluorescence of incubating medium was measured at 495 nm (excitation) and 520 nm (emission). To determine if the scaffolds support cell growth, we seeded bone marrow stromal cells (BMSC; ATCC, Manassas, VA) on the scaffolds and their proliferation and differentiation were investigated before and after apatite coating.

Results: Incorporation of CS or AG to CH scaffolds significantly decreased the pore size of scaffolds (figure 1a) from 128.6 μm (CH) to 91.9 μm (CH/CS) or 105.5 μm (CH/AG). The compressive modulus of the scaffolds were significantly increased from 2.1 MPa to 8.0 MPa or 5.6 MPa for CH/CS or CH/AG scaffolds, respectively, indicating strong ionic interaction between CH and CS or AG. The apatite coating on scaffolds exhibited plate-like morphology (figure 1a), and it appeared more homogenous on CH/CS than CH/AG or CH scaffolds. Distinctive phosphate (PO_4^{3-}) peaks were observed on the apatite-coated (Ap) scaffolds by FTIR analysis.

Initial burst release of histone was significantly higher than that of BSA from CH scaffold, indicating electrostatic repulsion between positively charged histone (pI = 10.8) and positively charged CH scaffold. In contrast, initial burst release of histone was significantly lower than that of BSA from CH/CS and CH/AG scaffolds indicating strong electrostatic interaction between histone and negatively charged CS or AG. Initial burst release of histone and BSA were significantly reduced on the Ap-scaffolds, which can be attributed to high protein retention capacity due to presence of plate-like morphology that provides more binding surfaces for non-specific protein absorption. The apatite coating enhanced cell spreading and proliferation of on the scaffolds (figure 1d). To investigate osteoinductivity of scaffolds, the picrosirius red was used to stain expressed collagen (figure 1e). The Ap-CH/CS completely covered by secreted collagen while expressed collagen observed mostly around cellular cluster for non-coated scaffolds.

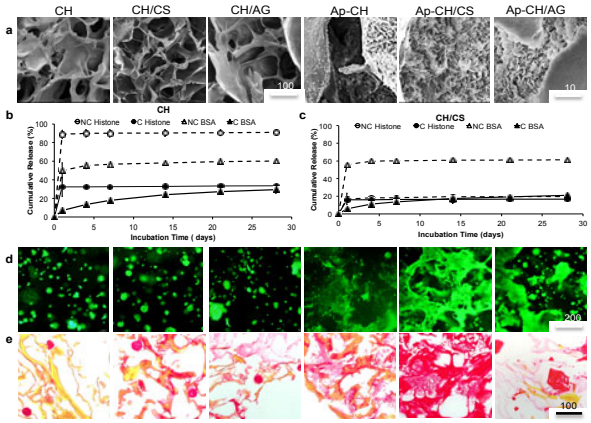

Figure 1. (a) SEM images. Release kinetic of (b) CH scaffold, and (c) CH/CS scaffold. (d) Calcian staining (e) picrosirius staining.

Conclusions: The uniform biomimetic apatite coating was obtained on CH/CS composite scaffold. The apatite coating provided a sustained release of loaded proteins as well as enhanced overall osteoinductivity of the scaffolds. Among experimental groups, Ap-CH/CS scaffold appeared to be the most promising system for promoting osteogenesis, and will be further evaluated in animal models to regenerate bone.

References:

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