**Self-healing Colloidal Gels are formed by Cohesive Interactions between Gelatin and Hydroxyapatite Nanoparticles**

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**Statement of Purpose:** Colloidal gels are a particularly attractive class of hydrogels which allow for a "bottom-up" fabrication of multifunctional biomaterials by employing micro- or nanoscale particles as building blocks to assemble into shape-specific bulk scaffolds. For applications in regenerative medicine, charged micro- or nanospheres made of biocompatible polymers are the most obvious candidate building blocks since the physicochemical properties of polymeric particles can be tailored to desire in terms of size, charge and chemical derivatization [1]. So far, however, the synthesis of colloidal composite gels composed of both organic and inorganic particles has not been explored yet. For regeneration of hard tissues, the introduction of inorganic building blocks such as calcium phosphate nanoparticles into colloidal gels could offer considerable advantages over purely organic colloidal gels by increasing stiffness, osteoconductivity and control over drug delivery. Therefore, we have investigated if colloidal gels can be formed by reversible, cohesive interactions between gelatin (Gel) and hydroxyapatite (HA) nanoparticles.

**Methods:** Gel nanospheres were prepared using using a two-step desolvation method, while HA nanoparticles were prepared using an established wet-chemical neutralization reaction between calcium hydroxide and phosphoric acid. To investigate the interactions between HA nanoparticles and Gel nanospheres, the self-assembly process of colloidal mixtures of Gel and HA nanoparticles was monitored at diluted conditions (~ 0.02 w/v%) using dynamic light scattering (DLS) and transmission electron microscopy (TEM) as a function of particle charge, HA to Gel ratio (HA/Gel ratio) and ionic strength, whereas the formation of colloidal composite gels at concentrated conditions was investigated using oscillatory rheometry as a function of solid content (up to 20 w/v%), HA/Gel ratio and ionic strength. Moreover, the biological performance of the resulting colloidal composite gels was studied in vitro by evaluating: i) gel degradation, ii) release kinetics of (radiolabelled) osteogenic protein BMP-2, and iii) cellular response of osteoblast-like cells cultured on colloidal composite gels.

**Results:** Characterization of dilute nanoparticle dispersions using DLS, TEM and sedimentation behavior confirmed that HA nanoparticles strongly aggregated with both cationic and anionic nanospheres, which suggested that the interaction forces between Gel and HA nanoparticles can be exploited to facilitate bottom-up synthesis of cohesive colloidal composite gels. To test this hypothesis, rheological characterizations of concentrated dispersions were performed which showed that colloidal Gel-HA composite gels can be formed by simple mixing of Gel and HA nanoparticles. We observed that small additions of HA nanoparticles (HA/Gel ratio=0.1) increased gel elasticity in a remarkably effective manner without compromising the beneficial self-healing behavior (70-80% recovery after destructive shearing) characteristic of colloidal gels made of gelatin nanospheres [2]. At a higher HA/Gel ratio of 1, the storage modulus G' of the colloidal composite gels increased to about 50 kPa, but at the expense of the self-healing capacity of the composite gels which dropped from 70% to less than 35%. This phenomenon can be explained by the lack of reversible interactions between HA nanoparticles. At even higher HA/Gel ratios, liquid-like behavior was observed similar to pure HA pastes without any self-healing capacity. Summarizing, HA/Gel ratios ranging from 0.1 to 0.5 were shown to be optimal by combining high elasticity with self-healing behavior. Moreover, it was shown in vitro that HA nanoparticles can be used as an additional tool to reduce the degradation rate of otherwise fast-degradable gelatin nanospheres and fine-tune the control over release of growth factors such as BMP-2 (Figure 1). Finally, it was shown that these colloidal composite gels supported attachment, spreading and proliferation of cultured stem cells.

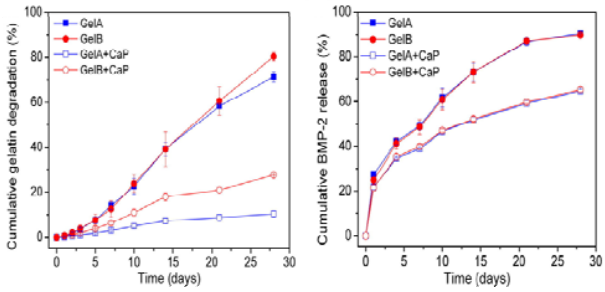


Figure 1: Cumulative degradation (left) and release of BMP-2 from colloidal gelatin/calcium phosphate gels

**Conclusions:** The current study has shown that organic-inorganic colloidal composite gels can be formed out of hydroxyapatite nanoparticles and gelatin nanospheres as building blocks. Depending on the ratio between Gel and HA nanoparticles, these novel colloidal Gel-HA composite gels exhibited a high gel elasticity, self-healing behavior, and gel stability at high ionic strengths without the need for chemical - potentially cytotoxic - functionalization to introduce sufficiently strong but reversible cohesive interactions. Moreover, it was shown in vitro that HA nanoparticles can be used as an additional tool to modulate the degradation rate and corresponding drug release behavior of colloidal gelatin gels.

**References:**

[1] Wang Q. Adv Mater 2008; 2008;20:236-239.
[2] Wang H. Adv Mater 2011;23:H119-H124.