Nanoengineered Bioactive Hydrogels for Cells-based Tissue Engineering

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Statement of Purpose: With an aging U.S. population, the occurrence of injuries and degenerative conditions are subsequently on the rise. As a direct result, there is an increase in demand for therapies that are able to repair damaged tissues and produce replacement organs. In particular, there is a great need for new bioactive materials that can direct stem cell differentiation and facilitate the formation of functional tissues. Recently, synthetic silicate nanoplatelets have shown promise in developing strong matrix, high-performance elastomers, super hydrophobic surfaces, super barrier thin films, flame retardant materials, mouldable hydrogels, hierarchical structures, and drug delivery devices. Although the above-mentioned reports have investigated synthetic silicates for a range of applications, the interaction of synthetic silicate nanomaterial with biological tissue at cellular levels has not yet been taken into consideration. Here, we show that addition of 2D silicate nanosheets to polymeric matrix enhances the physical and chemical properties of the nanocomposite network and also induces osteogenic differentiation of stem cells without using any growth factors.

Methods: The nanocomposite hydrogels were fabricated from methacrylated gelatin with varying of 2D silicate nanosheets (0.5, 1 and 2 wt%) along with 0.25% of photoinitator (IRGACURE 2959) (Fig. 1a). The prepolymer was crosslinked using UV radiation (6.9 mW/cm^2, 60 secs). The effect on addition of 2D silicate nanosheets to the polymer matrix was determined investigating various physical and chemical properties such as hydration degree, degradation rate, morphology, and mechanical stiffness. The effect of 2D silicate nanosheets on cellular adhesion, proliferation and differentiation was evaluated using human mesenchymal stem cells.

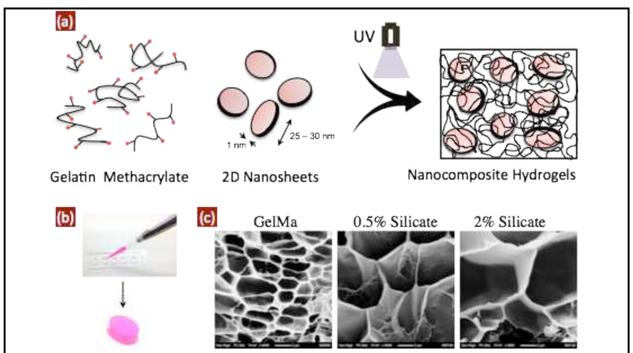

Figure 1: Synthesis of gelatin-silicate nanocomposite hydrogel.

Results: We have designed synthetic silicates loaded nanocomposite hydrogels to induce the osteogenic differentiation of human stem cells in growth factor-free microenvironment. 2D synthetic silicates are a novel class of ultrathin nanomaterials, with a high degree of anisotropy and functionality. Due to the anisotropic distribution of their surface charge, positive along the edge and negative on the top and bottom surfaces, the silicate nanoparticles can form self-assembled structures, which can dynamically form and break, creating shear thinning gels when in aqueous media. Upon UV exposure, self-assembled structures of gelatin methacrylate (GelMA) and synthetic silicate nanoparticles results in formation of highly crosslinked and interconnected network (Fig 1). The addition of silicates results in four-fold increase in compressive modulus along with an increase in pore size as compared to gelatin hydrogels (Figure 2a). *In vitro* tests indicated that the nanocomposites are cytocompatible and capable of promoting osteogenesis in absence of any osteoinductive factors as evident from alkaline phosphate (ALP) activity and production of mineralized matrix (Fig 2c&2d). Preliminary i*n vivo* biocompatibility of nanocomposite hydrogels investigated by dorsal subcutaneous injection in rats, indicate a normal locally restricted inflammatory reaction. Synthetic silicates are a novel class of ultrathin nanomaterials, with a high degree of anisotropy and functionality, which interact with biological entities in a substantially different manner than their respective 3D nano-, micro-, and macro- counter parts because of their high-surface-to-volume ratio.

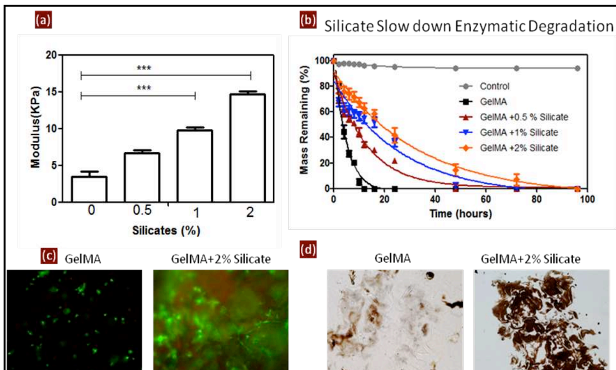

Figure 2: Addition of 2D silicate nanosheets (a) enhances mechanical stiffness and (b) reduces degradation rate. (c) 3D encapsulation of hMSCs showed enhanced cell adhesion and spreading due to addition of naosheets. (d) Formation of mineralized matrix is enhanced due to addition of nanosheets.

Conclusions: The nanocomposite hydrogels loaded with silicate nanoparticles show enhanced physical chemical and biological properties. The addition of silicate nanosheets show improved stiffness, *in vitro* stability, cell adhesion, viability and differentiation to hMSCs to osteogenic lineage.

References: (1) Gaharwar, A. K.; Peppas, N. A.; Khademhosseini, A. *Biotechnology and Bioengineering* **2014,** *111* (3), 441-453. (2) Gaharwar, A. K.; Mihaila, S. M.; Swami, A.; Patel, A.; Sant, S.; Reis, R. L.; Marques, A. P.; Gomes, M. E.; Khademhosseini, A. *Adv Mater* **2013**.