Protein stabilized and sustained deliverable nanofiber '*smart scaffold*' for multiphase tissue regeneration
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Statement of Purpose: Tissue engineering (TE) represent a paradigm shift in healthcare therapies and treatments by repairing, replacing, or regenerating damaged cells and tissues in human body. Chemical cues such as growth factors (GFs) and cytokines, and there successful delivery is the key components in TE with the ability to target specific tissue regeneration. Current techniques of immobilization and loading of GF into a polymer matrix (using emulsion method) have shown only limited success in TE field due to the loss of GF's bioactivity during fabrication process and storage, as well as generally low encapsulation efficiency, and uncontrolled release profiles.[1] Thus it is difficult to provide artificial tissue microenvironment for cell growth and specific tissue differentiation. A number of approaches have been developed to ameliorate the impact of individual processing stresses, but no single approach has been available heretofore which would overcome these challenges of GF delivery from scaffold. Far from meeting this ideal, many approaches for improving one aspect of performance are neutral or deleterious to others.[2]

We developed novel nanoencapsulation of protein in to sugar-glass-matrix to form protein-sugar-glass-nanoparticles (SGnP) system to overcome these formidable challenges of GF (e.g., protein) stabilization and their sustained delivery (Fig. 1). SGnP could be used develop a generic platform for GF encapsulated '*smart scaffold*' of any polymer and protein system for functional tissues regeneration by protecting protein's activity lost against processing and storage stresses, while reaching high loads and exhibiting burst-free sustained release of protein in their active form to provide suitable tissue microenvironment for stem cell differentiation .

Methods: We have encapsulated different GFs (for bone BMP 2 and cartilage BMP 7+TGFβ) into SGnP by flash-freezing and freeze-dried method reported in Ref.3. GF-SGnP (1-2 % (w/w)) is then suspended in organic solvent-based polymer (PCL/PLGA) solutions, from which tissue scaffolds were fabricated by electrospinning. Four different scaffolds are fabricated with/without encapsulating specific GF for control (no GF), bone (BMP 2), cartilage (BMP 7+ TGFβ) and bone-cartilage interfacial tissues (BMP 2 and BMP 7+ TGFβ). Protein release profile from scaffolds is also studied to check their burst free sustain release. We evaluated the performance of these *smart scaffolds* for specific *in vitro* tissue regeneration using hBMSC and cultured for 30 days using normal growth media. Gene expression, protein assay and staining are used to characterize the differentiation.

Results: We have reported that SGnP system provide excellent protection of GF from array of non-aqueous
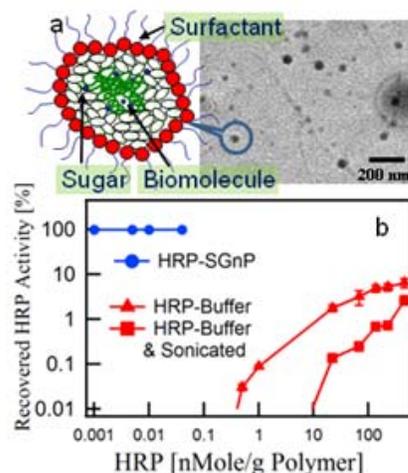

Fig. 1 (a) Schematic and TEM of SGnP system and (b) effectiveness on protecting biomolecules from process stresses compared common approach where horseradish peroxidase (HRP) is added from buffer into chloroform + poly-caprolactone; PCL solution
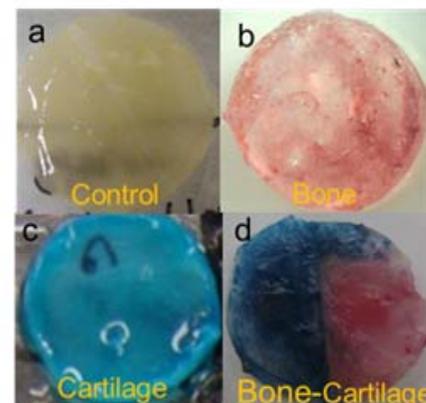

Figure 2. In vivo tissue regeneration using smart nanofiber scaffold where GF are encapsulated using SGnP system. (a) control scaffold without GF (b) bone formation on BMP 2-deliverable scaffold (alizarin red staining), (c) cartilage formation on TGF-β/BMP 7 deliverable scaffold (toluidie blue staining), (d) cartilage-bone and their interfacial tissues on a composite scaffold providing different GF microenvironment

solvents with essentially no activity loss after exposure to solvents with polarity index ≤ 5, as compared to > 90% loss (and up to 99.97% loss) using emulsion methods commonly in use.[3] The PCL or/and PLGA nanofiber scaffold shows sustained release profiles of GF from scaffold without significant burst release (<5 % release within day 1) as SGnP system provides excellent dispersion of encapsulated protein in the polymer matrix and consequently their diffusion based release. After 21 days of culture of stem cells (hBMSC) on these *smart scaffolds* in a 48 (single GF loaded scaffold) and 12 (composite scaffold) well plate using normal media, we observed formation of bone, cartilage as well as bone-cartilage interfacial tissues in a single scaffold (Figure 2).

Conclusions: SGnP is used to develop GF encapsulated '*smart scaffold*' system for different functional tissues regeneration. The microenvironment of GF is essential for generate specific tissues than the global concentration of GF i.e., concentration in culture media. Thus it is possible to generate suitable microenvironment of GF in a single scaffold to generate multiple interfacial tissues using the *smart scaffold* system where GFs are encapsulated and stabilized within SGnP system.

Reference: [1] K. Fu, A. M. Klibanov, R. Langer, *Nat. Biotechnol. 18*, 24. 2000. [2]. C. Perez, at el, *Journal of Pharmacy and Pharmacology 54*, 301. 2002. [3] J. Giri at el, Advanced Material *23*, 4861, 2011. [3] J. C. Sy, A. S. Klemm, V. P. Shastri, *Advanced Materials 21*, 1814, 2009.