Human-based nanocomposite cryogels for hemostatic and wound healing applications

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Statement of Purpose: In trauma surgery, a fast and effective hemostatic agent is crucial to prevent death. The current used hemostatic sponges are highly effective in stopping the hemorrhages, however they have a limited stability, shape memory, and biological functionality to induce an efficient regenerative healing after injury[1-2]. Blood derivatives have attracted great attention as an inexpensive milieu of bioactive molecules (e.g., growth factors, cytokines), self-assembling scaffolding proteins (e.g., fibrinogen, fibronectin, vitronectin), and antimicrobial peptides (e.g., platelet factor-4) that have the ability to enhance angiogenesis, stem cell recruitment, and tissue regeneration[3]. Among those, platelet lysate (PL) has attracted great attention as a milieu of supra-physiological doses of biomolecules that can be easily standardized. However, the current PL scaffolds showed limited stability and weak mechanical strength, which severely limits its performance as a bioinstructive and hemostatic biomaterial. Herein, we propose the use of aldehyde-functionalized CNC (a-CNC) that will be crosslinked through reversible Schiff base bonds established with the amine groups of PL proteins to produce a stable hemostatic cryogel for wound healing applications.

Methods: PL was prepared from a pool of ten donors in sterilized conditions. PL-CNC cryogels were obtained using a simple double barrel injection system (1:1 ratio from Medmix, Switzerland) filled with PL in one compartment and with a-CNC water dispersions (1.22 to 2.44 wt.%) in the other. After extrusion/casting into square molds, the cryogel precursors were placed in a freezer at -80ºC and subsequently in the freeze-dryer until full cryogelation. The developed cyogels were characterized regarding their physicochemical and hemostatic properties as well as their *in vitro* biological performance.

Results: PL cryogels (PL-CNC 0) solubilized immediately after immersion in PBS while CNC incorporation (0.61 to 1.2 wt.%) as a strengthening crosslinker improved PL cryogels stability. Increasing CNC loadings gradually slow the PL-CNC cryogel degradation rate and increased their specific modulus without the loss of the intrinsic PL bioactive properties. Interestingly, PL-CNC cryogels showed a reversible deformation at over 70% strain level and a fast shape recovery (seconds), which enable their clinical application using minimal invasive strategies (e.g. injectable shape memory cryogel). After blood immersion, the PL-CNC cryogels absorb comparable volumes of blood at a faster rate than commercial gelatin-based hemostatic sponges, along with better maintenance of the original shape and structural integrity (Figure 1) due to their inherent interconnected macro-porous structure (90 vol.%) and high elastic nature. Moreover, nanocomposite cryogels biophysical (e.g. porous microstructure) and biochemical (e.g. growth factors and cytokines) cues allowed human adipose-derived stem cells (hASCs) cell survival and proliferation, which also had an impact on other important cell behaviors such as cell migration.
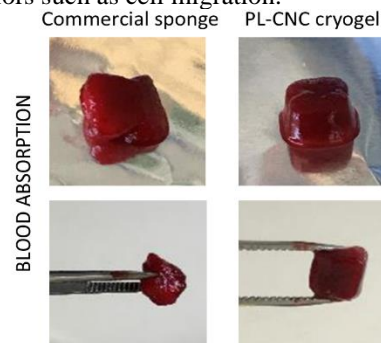

Figure 1. Blood adsorption capacity and shape maintenance of PL-CNC cryogels compared to commercial gelatin-based hemostatic sponges.

Conclusions: The developed human-based nanocomposite cryogels showed similar hemostatic performance and lower shrinkage as compared to commercial gelatin hemostatic sponges. Moreover, the developed cyrogels exhibited tunable biophysical and biochemical properties that favor the cell supportive cryogel properties. In summary, the proposed PL-CNC cryogels allow the use of PL not only as a source of signalling biological factors involved in wound healing, but also as a user-friendly off-the-shelf hemostatic biomaterial in minimal invasive strategies to promote regenerative wound healing outcomes.

References: [1]Kauvar, DS et al. J Trauma. 2006; 60:S3-S11. [2]Landsman, TL et al. Acta Biomaterilia. 2017; 47:91-99. [3]Mendes, BB et al. Adv Drug Deliv Rev. 2017; 129:376-393.