Introduction: Collagen hydrogels have been widely investigated as scaffolds for vascular tissue engineering due in part to the abundance of collagen in the vessel wall and the capacity of collagen networks to support cell spreading. However, collagen hydrogels display relatively low stiffness and strength, have a tendency to undergo rapid degradation, and are highly susceptible to cell-mediated contraction. In the current work, we develop and characterize a hybrid natural-synthetic interpenetrating network (IPN) that retains the benefits of collagen in terms of enabling robust cell elongation, but which displays enhanced stiffness and strength as well as increased resistance to cell-mediated contraction and degradation. Toward this end, we first formed a collagen-based hydrogel, infused this hydrogel with a solution of poly(ethylene glycol) diacrylate (PEGDA) plus photoinitiator, and subsequently crosslinked the infusate by exposure to longwave UV light. This approach allowed for the tailoring of scaffold mechanical performance by varying the molecular weight of the infusing PEGDA, and resulted in networks which displayed the resistance of PEGDA to degradation and contraction. Furthermore, we demonstrated the ability of these collagen I-PEGDA (coll-PEGDA) IPNs to support the differentiation of encapsulated human mesenchymal stem cells (MSCs) toward a vascular smooth muscle cell (VSMC) fate.

Materials and Methods: *Fabrication of Coll-PEGDA IPN*. Collagen hydrogels (3 mg/mL) were formed at 37 ºC. These collagen hydrogels were then infused with a solution of 11 wt% PEGDA (3.4 kDa, 6.0 kDa and 10.0 kDa) and Irgacure 2959 as photoinitiator, and subsequently crosslinked by exposure to longwave UV light for 6 min. Rheological and tensile properties were evaluated for each IPN formulation. The micro- and nano-structures of the IPNs were also characterized by laser reflection confocal and scanning electron microscopy (SEM). *Construct Culture and Analysis*. Human bone-marrow derived MSCs (Lonza) were encapsulated at $1x10^6$ cells/cm^3 within the collagen hydrogel prior to 6.0 kDa PEGDA network formation. Resulting constructs were cultured for 2 wks in DMEM with 10% MSC-qualified FBS supplemented with 10 ng/mL of TGF-β_3 and 1% antibiotic/antimycotic. Gene expression was then analyzed relative to GAPDH using qRT-PCR.

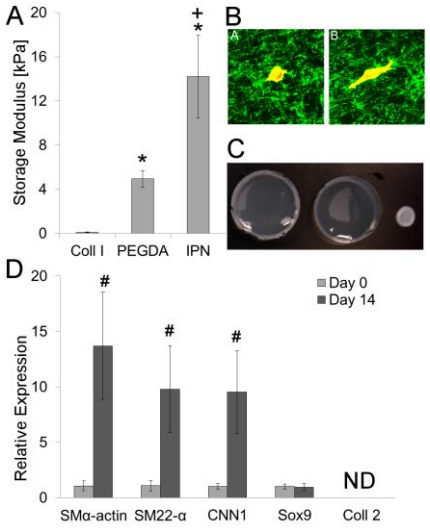


Fig. 1. (A) Storage modulus of Coll-PEGDA IPNs relative to pure PEGDA and pure collagen counterparts. (B) Control over extent of cell spreading within the IPN networks (collagen fibers - green, cells - yellow). (C) Size of Coll-PEGDA IPN, PEGDA, and collagen hydrogels following 2 wks of culture. (D) Relative gene expression of VSMC and chondrogenic markers in Coll-PEGDA IPNs in media supplemented with TGFβ_3.. *, $^+$, significantly different from pure collagen and PEGDA; # significantly different from day zero

Results and Discussion: For a 11 wt% 3.4 kDa PEGDA infusate, the formation of a coll-PEGDA IPN resulted in 140-fold increase in elastic modulus relative to the pure collagen counterpart and a nearly 10-fold increase in modulus relative to the corresponding pure PEGDA gel (**Fig. 1A**). The coll-PEGDA IPNs also exhibited tensile strengths ranging from 50-110 kPa, depending on the MW of PEGDA utilized (data not shown). These tensile strengths were approximately 2-fold greater than the corresponding pure PEGDA gels and markedly greater than that of pure collagen gels (< 1 kPa tensile strength). In addition, these IPN systems allowed for high viability (> 90%) cell encapsulation via the inclusion of cells within the collagen hydrogel precursor solution (data not shown). By varying the time between the formation of the cell-laden collagen hydrogel and the formation of the PEGDA secondary network, the degree of cell elongation within the IPN could be controlled from round to highly elongated (**Fig. 1B**). Finally, the capacity of Coll-PEGDA IPNs to support MSC smooth muscle progression was analyzed following 2 wks of cell culture in the presence of TGF-β_3, a growth factor which can stimulate VSMC or chondrogenic differentiation. Importantly, elongated MSCs within the coll-PEGDA networks showed up to a 14-fold increase in VSMC marker expression relative to day 0, whereas no increase in chondrogenic markers was observed ($p < 0.05$; **Fig 1D**).

Conclusions: Coll-PEGDA IPNs fabricated via sequential infiltration have significant potential in vascular graft applications due their ability to support smooth muscle lineage progression and their enhanced stiffness and strength and increased resistance to cell-mediated contraction (**Fig. 1C**) relative to pure collagen.

Acknowledgements: We gratefully acknowledge funding from the NIBIB.