A Multilayer Hydrogel of Unique Material Compositions Direct a Single Progenitor Cell Population into Zonally Organized and Mechanically Relevant Articular Cartilage

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Statement of Purpose: Despite significant advances in cartilage tissue engineering, differentiating stem cells into zonally organized, native-like, articular cartilage with spatially-varying mechanical properties and extra-cellular matrix (ECM) composition, has not yet been achieved. We show, for the first time, that unique combinations of synthetic and natural biopolymers "direct" marrow stem cells (MSCs) to differentiate into superficial, transitional, and deep zones of articular cartilage. Specifically, our previous work indicated that incorporating chondroitin sulfate (CS) and matrix metalloproteinase-sensitive peptides (MMP-pep) into PEG hydrogels (PEG:CS:MMP) induced high levels of collagen II, low levels of proteoglycans and low compressive modulus, similar to the superficial zone. PEG:CS hydrogels produced intermediate-levels of collagen II and proteoglycans, like the transitional zone, while PEG:hyaluronic acid (HA) hydrogels induced high proteoglycan and low collagen II levels leading to high compressive modulus, similar to the deep zone [1]. **Fig. 1** illustrates the correlation of the chondrogenic phenotype produced by the specific biomaterial compositions. Here we show that creating a multi-layered scaffold, incorporating these zone-specific biomaterials, allowed MSCs to differentiate into zonally-organized articular cartilage with spatially varying biochemical and mechanical properties.

	Collagen X			Collagen II			GAG Content		
Hydrogel Groups	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
PEG:CS:MMP	26	45	54	99	65	36	53	98	56
PEG:CS	10	16	24	59	55	24	99	137	80
PEG:HA	8	32	24	26	31	16	176	236	132
CS	49	96	65	45	26	11	89	265	308

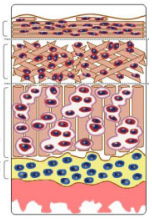

Figure 1. Correlation of cartilage generated in various hydrogel compositions to the specific zones of articular cartilage. (Adopted from Poole et al.)

Methods: Chondroitin sulfate (CS) and hyaluronic acid (HA) was acrylated using glycidyl methacrylate. The protocol was adopted from Varghese et al. [2] and Leach et al. [3], respectively. An MMP-sensitive (QPQGLAK) peptide was synthesized and modified by adding acryl groups to amine group of the N terminal and to the amine group on the lysine. The multi-layered hydrogels were fabricated from the bottom layer up. First 100 µL of PEG:HA-MSC mixture was polymerized under UV for 3 minutes to represent the deep zone then we added 100 µL of PEG:CS-MSC mixture on top of the partially polymerized bottom layer and again was polymerized under UV for an additional 3 minutes to represent the transitional zone. Finally, for the superficial zone 100 µL of PEG:CS:MMP was added and the entire hydrogel was fully polymerized for 5 minutes using a long-wave ultraviolet lamp (Blak-Ray) at an intensity of ~10 mW/cm^2. The hydrogel constructs were cultured in chondrogenic medium containing 1% penicillin-streptomycin, no FBS, and 10ng/uL TGF-β1 for 2, 4 and 6 weeks

Results: Cartilage synthesis was determined by gene expression of collagen II and X within the hydrogel constructs at 2, 4 and 6 weeks. The collagen II expression decrease from the superficial to the deep zone at all time points as shown in **Fig. 2A**. Collagen X expression was initially the same between all layers at 2 weeks, but began to increase from the superficial to the deep zone at 4 and 6 weeks as shown in **Fig. 2B**. At all time points, both the GAG content (**Fig. 2C**) and mechanical strength (**Fig. 2D**) exhibit similar trends to native articular cartilage, increasing from the superficial to the deep zone. These results demonstrate that zonally organized cartilage-tissue can be achieved from a single stem cell population using unique and spatially varying biomaterial compositions.

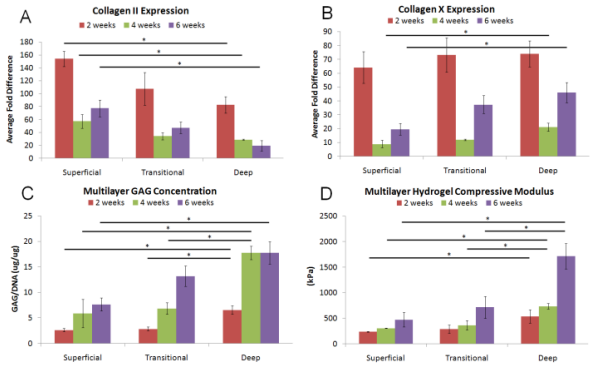

Figure 2. Multilayer gene expression of (A) collagen II, (B) Collagen X (C) GAG concentration and (D) compressive modulus.

Conclusions: In summary, we demonstrated that spatially varying biomaterial compositions within a multilayer hydrogel can be used to induce differentiation of MSCs into a single, zonally-organized 3D articular cartilage-like tissue. The ability to create native-like, mechanically relevant articular cartilage consisting of zone specific layers provides a new direction in cartilage tissue engineering and could be invaluable for cartilage repair if incorporated with current minimally invasive surgical techniques. Additionally, this technique represents a fundamental advance over current technologies in regenerative medicine, moving away from attempting to create homogenous tissue structures matching bulk properties, to creating more physiologically and functionally relevant tissue substitutes with native-like, spatially varying properties.

References:

1. Nguyen, H.L., et al., Biomaterials, 2010. Nov. 12 Epub
2. Varghese, S., et al., Matrix Biol, 2008. **27**(1): p. 12-21.
3. Leach, B.J.,et al., Biotechnol Bioeng, 2003. **82**(5): p. 578-89.