Ascorbic Acid Promotes Valve Interstitial Cell-mediated Extracellular Matrix Deposition in Hydrogel Scaffolds

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Statement of Purpose: Current options for aortic valve replacements are non-viable and thus lack the ability to grow and remodel. Hence, pediatric and adolescent patients often require multiple valve replacement operations to implant larger valve substitutes as they grow. An ideal valve substitute for these patients should be living and dynamic, mimicking the composition and function of the native valve. Our current work aims to investigate the potential of cell-laden, proteolytically degradable poly(ethylene glycol) (PEG) diacrylate hydrogels to construct living valve substitutes that can grow and remodel. A significant challenge for valve substitutes is to recapitulate the natural extracellular matrix (ECM) of valve leaflets, including collagens (Col), elastin, proteoglycans, and glycosaminoglycans, since the ECM is largely responsible for the unique mechanical properties of the valve tissue. Valvular interstitial cells (VICs)—the predominant cell population in valve leaflets—are responsible for active ECM synthesis in the valve tissue. These cells were isolated and encapsulated within hydrogels in an effort to study their ECM deposition in 3D scaffolds *in vitro*. Moreover, ascorbic acid (AA) has been reported to promote collagen secretion of smooth muscle cells and fibroblasts [1-2]. However, there is little work on its effect on VICs. In this work, the effect of ascorbic acid (AA) treatment on VIC Col secretion and phenotype marker expression was investigated.

Methods: Primary VICs were harvested from porcine aortic valves and encapsulated within matrix metalloprotease (MMP)-sensitive, cell-adhesive PEG hydrogels at a density of 1×10^7 cells/ml. Briefly, the MMP-2-sensitive peptide sequence GGGPQGIWGQGK (PQ) was incorporated into the backbone of a PEG diacrylate polymer while the adhesion ligand RGDS was conjugated to PEG-monoacrylate to allow grafting into the hydrogel network. 4 wt% PEG-PQ-PEG with 5 mM PEG-RGDS was photocrosslinked by white light exposure (25s) using 10 μM eosin Y as the photoinitiator. The cell-laden hydrogels were cultured in growth media (GM) (DMEM and F12 1:1, 1.6% HEPES, 1% Hyclone antibiotic–antimycotic solution, 1% Hyclone bovine growth serum) or GM supplemented with 50 μg/mL AA (GM+AA) for 28 days. Cell viability was assessed by calcein AM and ethidium homodimer staining (Live/Dead kit). MMP secretion by encapsulated VICs was determined by gelatin zymography of the cell culture supernatants. Cell-laden hydrogels were immunohistochemically stained and imaged via confocal microscopy to assess cell morphology, proliferation, phenotype, and Col deposition within the hydrogels.

Results: Approximately 98% cells were viable after encapsulation for 3 days. 56±12% of VICs were positive for the proliferation marker ki67 at day 30, indicating ongoing growth of the engineered tissue. Zymography showed that VICs encapsulated within PEG hydrogels secreted MMP-2. As a result, VICs spread well within MMP-2 labile hydrogels (Fig. 1A). Encapsulated VICs also secreted Col I and III, which increased with culture time and tended to be organized into Col fibrils along cell spreading direction (day 28, Fig. 1A, day 14, 21 not shown). AA treatment significantly increased Col I and III secretion by 113% and 221% respectively at day 28 (Fig. 1A-C). The results of α-smooth muscle actin (α-SMA) staining and alkaline phosphatase (ALP) staining demonstrated that AA treatment did not significantly influence the expression of these phenotype markers.

Conclusions: MMP-sensitive, cell-adhesive PEG hydrogels supported VICs' growth and proliferation in 3D for up to 30 days, showing the utility of these scaffold materials for long-term culture of VICs. Moreover, encapsulated VICs actively degraded the hydrogels and secreted Col I and III. We also demonstrate that AA promoted Col I and III secretion of VICs without negatively influencing the expression of cell phenotype markers. While the Col promotion effect is promising, the later is also important because VIC phenotype changes could cause pathological progression of valve diseases. Hence, AA promotes VIC-mediated ECM remodeling, and is potentially beneficial for the formation of living valve substitutes. Future work focuses on further promoting the maturation of the cell-laden hydrogels by optimizing the mechanical and biochemical cues from the hydrogels, such as substrate rigidity and growth factors.

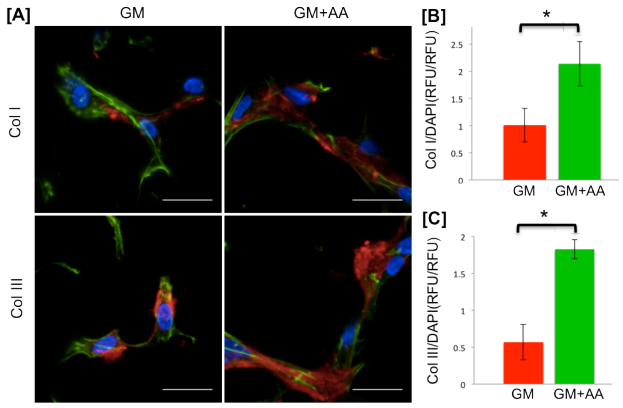

Fig 1. Col I and III secretion of VICs encapsulated in PEG hydrogels for 28 days in GM or GM+AA. [A] Fluorescent images: red (Col I or III), blue (DAPI, nuclei), green (phalloidin, actin). Scale bars=20μm. Z-projection covering a depth of 80μm. [B-C] Quantitative analysis based on relative fluorescence intensities of Col I, Col III and DAPI by Image J. * $p<0.001$ by students' t-test, n=8.

References: 1. Qiao, H. J Vasc Res. 2009; 46:15-24.
2. Abe, T. J Cell Physio. 2001; 189: 144-151.