MMP-I UP-REGULATION AS A POTENTIAL MECHANISM FOR INCREASED COMPLIANCE IN MUSCLE-DERIVED STEM CELL-SEEDED SIS FOR UROLOGIC TISSUE ENGINEERING

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INTRODUCTION

Porcine small intestinal submucosa (SIS, Cook Biotech) can be used as a sub-urethral sling device for treatment of Stress Urinary Incontinence (SUI). However, SIS, along with other biologically derived sling materials, has the tendency to degrade and lose mechanical integrity in vivo over time. In order to improve integrity and function, SIS may be seed with cells. Previously, we demonstrated that SIS seeded with MDSC formed a calcium-dependent contractile muscle-like tissue after 4 weeks of culture(1). We have also shown that MDSC seeded onto SIS increase the mechanical compliance of SIS (2). To date, however, the mechanisms by which the MDSC/SIS construct becomes more compliant are yet to be elucidated.

One possible mechanism responsible for an increase in compliance is the release of matrix metalloproteinase-I (MMP-I), which degrades collagen type-I, the main component of SIS. We hypothesize that the release of MMPs and break down in collagen fibers by MDSCs seeded onto SIS is a potential mechanism responsible for a change in the mechanical properties of MDSC/SIS compared to unseeded SIS. In the present study, we examined the impact of MMP-I on the mechanical properties and surface characteristics of SIS as well as the MMP-I activity of MDSC seeded onto SIS .

METHODS

Samples of SIS (Cook Biotech) were cut into circles and attached with an o-ring to a modified costar transwell cell culture insert (Fisher Scientific) such that the growth area of the SIS was 4.7 cm^2. SIS samples in the constructs were placed in 6 well tissue culture dishes. Mc13 cells (murine, transfected with a plasmid encoding for the b-galactosidase for the tracking purpose) passages 8-10, were seeded at $1x10^6$ cells/ insert in 5.5 mL of in DMEM supplemented with 20% FBS, 1% penicillin streptomycin, and 500 µg/mL G418. Media were changed every 48 hours and supernatant and cell lysates were collected at time points of 1, 3, 5, 7, and 10 days for assays.In order to examine cell proliferation, a PicoGreen dsDNA quantitation kit (Molecular Probes, Eugene, OR) was used on cell lysates. MMP-I activity of MDSC on SIS in culture media at 1, 3, 5, 7, and 10 days was determined using a collagenase assay kit (Chondrex Inc.). The data were normalized by total protein concentration, which was determined using Coomassie Blue total protein kit (Fisher Scientific) as per the manufacturer's instructions.

Twenty samples of SIS were cut in 1 cm^2 squares, sterilized in 70% EtOH, and rehydrated in HBSS for 2 hours. Sixteen of the twenty samples were treated with culture media containing 0.16 U/mL collagenase type I (an equivalent amount of collagenase activity as found at day 1 in the MDSC/SIS seeded samples) in DMEM for 3, 4.5, 5, and 24 hours. The enzyme reaction was stopped in 0.5 mM EDTA for 10 minutes. Four samples of SIS were treated in culture media not containing collagenase-I for 24 hours were used as a control. Biaxial testing was performed on all samples as described previously(2).

Six samples of MDSC/SIS and SIS digested in collagenase-I at varying time points were fixed in 2.5% gluteraldehyde, rinsed in PBS, soaked in 1% OsO4, and dehydrated in EtOH. Samples underwent critical point drying and sputter coating. Then, samples were viewed with a JSM6330F Scanning Electron Microscope in order examine initial cell attachment and surface degradation.

RESULTS AND DISCUSSION

The results of biaxial mechanical testing provide evidence that digestion of SIS with collagenase-I for 5 hours increased up to 7%. This increase in compliance was statistically similar ($p=0.356$) to the compliance of MDSC seeded SIS at 10 days.

Furthermore, DNA quantification showed that the DNA content of MDSC/SIS increased over time indicating that SIS supports cell growth and proliferation. The MMP assay revealed that there was a significant amount ($p<0.05$) of MMP-I present in supernatant from soaked, unseeded SIS compared to media alone. Additionally, MMP-I activity of the MDSC seeded SIS was significantly higher ($p<0.0025$) after one day in culture compared to samples collected from subsequent time points and the unseeded control. These results suggest that the MDSC synthesize and release MMP-1 into the ECM to digest the SIS substrate, which may allow the cells to penetrate and to integrate into the substrate.

CONCLUSIONS

The increase in mechanical compliance seen in MDSC seeded SIS may be mimicked with collagenase-I digestion. MDSC initially respond to SIS by releasing MMP-I, as exhibited through biological assays. The released MMP-I subsequently breaks down the collagen fibers in the SIS. The break down of collagen fibers, in turn may lead to the increase in compliance seen in the MDSC seeded SIS.

REFERENCES

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ACKNOWLEDGEMENTS

Source of funding: NIH AR-49398
The authors would like to thank Cook Biotech and Cook MyoSite for materials and technical assistance.