ENHANCING MECHANICAL PROPERTIES OF ENGINEERED TISSUES VIA LYSYL OXIDASE CROSSLINKING OF THE ECM

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INTRODUCTION

A number of strategies have been investigated to enhance the mechanical stability of engineered tissues. In this report, we utilized lysyl oxidase (LO) to enzymatically crosslink extracellular matrix (ECM) proteins, particularly collagen and elastin, to enhance the mechanical integrity of the ECM and thereby impart mechanical strength to the engineered tissue. Vascular smooth muscle cells genetically engineered to produce LO were incorporated into collagen gels or polyethylene glycol diacrylate hydrogels (PEG-DA) and subjected to dynamic tensile testing after in vitro culture. This novel strategy successfully demonstrated the enhancement of tissue engineered constructs' mechanical properties.

MATERIALS AND METHODS

LO Transfection of Cells

VSMCs were seeded at 7,000 cells/cm^2 prior to day of transfection. The cells were transfected with a plasmid construct (pcDNA3, Invitrogen) harboring the LO gene using a commercially available transfection reagent (Fugene, Roche) according to the manufacturer's instructions. As a control, mock wells received the plasmid DNA vector with no LO cDNA. Some wells were treated with β–aminoproprionitrile (BAPN, 300 μg/ml), LO inhibitor, as a control.

Desmosine Quantitation

Desmosine content was determined via radioimmunoassay as previously described[1].

Mechanical Testing of Collagen Gels

Collagen gels were fabricated from a sterile solution of pepsin-solubilized bovine dermal collagen dissolved in 0.012 N HCl that gels upon pH neutralization. The soluble form of collagen (3 mg/ml, Vitrogen) was mixed with 10x phosphate-buffered saline solution and NaOH (0.1 N) at 8:1:1 ratio respectively. Cells were mixed with the collagen solution and then aliquoted into 6-well plates. Collagen gelation was initiated by placing the seeded gels in a 37° C dry incubator and absence of CO_2. After gelation, complete media was added and the seeded gels were moved to cell culture incubator where they were cultured for 3 weeks. BAPN was added to some wells as a control. The collagen gels were attached to balsa wood using a cyanoacrylate glue to mediate the clamping of gels for tensile testing. Tensile testing was performed with a strain rate of 100 μm/second using a 150 g loading cell. Elastic moduli and tensile strengths were determined from stress-strain plots.

PEG-DA Hydrogel Synthesis

PEG-DA polymer was prepared as previously described[2]. Hydrogels were prepared by dissolving the PEG-DA in 10 mM HEPES-buffered saline (pH7.4) and an equal

volume of a SMC-suspension at $4x10^6$ was added to the aqueous polymer solution. 5 µl/ml of 2,2-dimethyl-2-phenyl-acetophenone in N-vinylpyrrolidone (600 mg/ml) was then added and 2 ml of the solution was placed in a rectangular–shaped mold (3mm thickness, 13 mm width). This was then exposed to UV light (365 nm, 10 mW/cm^2) for 30 s and the hydrogels were then cultured in medium similarly to collagen gels. The hydrogels were subjected to dynamic tensile testing after 3 weeks in culture.

RESULTS

Vascular smooth muscle cells (VSMCs) were liposomally transfected with the LO gene. Western Analysis confirmed increased LO expression (data not shown) and increased LO activity was demonstrated as increased levels of desmosine, a product of LO crosslinking in the ECM (Figure 1).

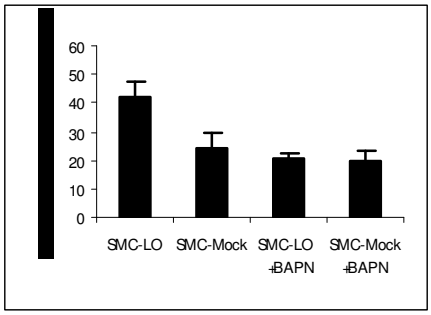

Figure 1: Desmosine levels in LO-or mock-SMCs cultures in the presence or absence of the LO inhibitor BAPN ($p<0.001$, n=6).

The effects of altered ECM crosslinking on mechanical properties was first evaluated in collagen gels. LO- or mock-SMCs were seeded into collagen gels and cultured for 3 weeks. We started with this tissue engineering model to assess the effects of LO in the presence of its natural target, collagen. Collagen gels seeded with LO-transfected SMCs had an elastic modulus approximately 75 kPa while controls and BAPN-treated samples were at or below 40 kPa (figure2). Ultimate strength was increased as well, with LO-transfectants having approximately double the strength of mock controls (figure 2).

The effects of ECM crosslinking on mechanical properties was further explored in a different model, a potential scaffold material for tissue engineered vascular grafts. PEG-DA hydrogels seeded with LO-transfected SMCs and cultured for 3 weeks had an elastic modulus of 85 kPa while controls had 35 kPa (figure 3). Similarly, the ultimate tensile strength of gels seeded with LO-SMCs was 13 kPa compared to 7 kPa of controls (figure 3).

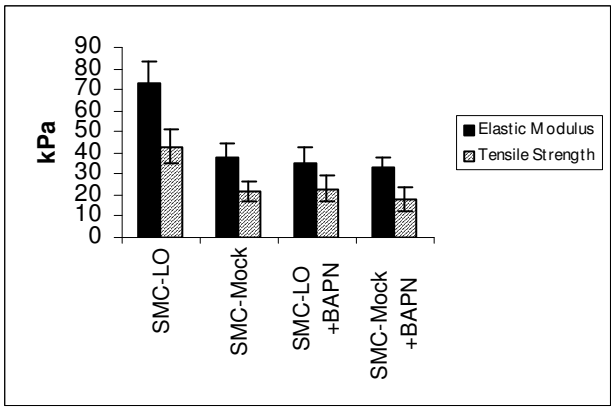

Fig. 2: Mechanical properties of collagen gels seeded with transfected SMCs in the presence or absence of BAPN ($p<0.03$, n=5).

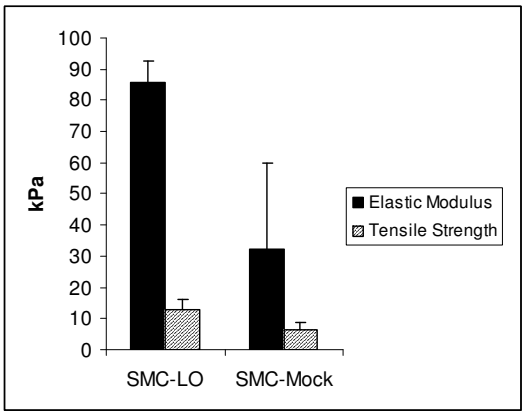

Figure 3: Mechanical properties of PEG-DA hydrogels seeded with transfected SMCs and cultured for 3 weeks ($p<0.02$ for elastic modulus, n=4).

The effect of elevating LO activity on SMC proliferation was determined in order to isolate the source of mechanical properties. The proliferation of transformed SMCs was similar throughout day 8 (not shown), thus attributing ECM crosslinking to increased mechanical properties.

ACKNOWLEDGEMENTS

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REFERENCES

[1]. Conn Tiss Res 33 (1995) 133-140.
[2]. Biomaterial 22 (2000) 3045-3051.