Trimethylamine N-oxide enhances the mechanical and biochemical properties of tissue engineered cartilage

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Introduction

Sharks have a skeletal structure of cartilage instead of bone. Urea and trimethylamine N-oxide (TMAO) is found in their blood and tissues and play a role in osmo-regulation, preventing sharks from undergoing dehydration in their seawater environment. TMAO is thought to counteract the effects of urea by stabilizing protein folding in shark tissue [1-2]. Inspired by the role that TMAO plays in the cartilaginous shark, this study investigates the effects of TMAO and urea on mechanical and biochemical properties of engineered cartilage constructs. We hypothesize that TMAO can serve as a novel media supplement to promote *in vitro* development of engineered cartilage.

Methods

Articular chondrocytes were harvested from juvenile bovine wrist joints (2-4 weeks old), and digested for 8 hrs at 37ºC with Collagenase V (Sigma). The cells were expanded for one passage in DMEM media containing 10% FBS, 1% PSAM, 5 ng/ml bFGF, 10 ng/ml PDGF, and 1 ng/ml of TGF-b1 (plating density = 180 cells/cm^2) [3]. The passaged cells were seeded in 2% w/v agarose at a concentration of 30M cells/ml (construct dimensions: ϕ = 4mm, thickness = 2.34mm). Two studies were performed to assess the influence of TMAO on development of engineered cartilage:

Study 1: Six culture medias were evaluated, with serum free media containing ascorbic acid and 10ng/ml of TGF-b3 for the first 14 days used as the control [4]. TMAO was added to the media at concentrations of 55mM and180mM, and urea was added to the media at concentrations of 90mM and 360mM. The final group included a combination (combo) of TMAO (180mM) and urea (360mM).

Study 2: Digestion of mature tissue engineered constructs with chondroitinase (cABC) temporarily suppresses the glycosaminoglycan (GAG) content, increases the collagen content and improves the mechanical properties [5]. Constructs in chondrogenic media (CM) were digested with 0.15U/mL cABC (Sigma) for 48 hours. Following the digestion, constructs were cultured in either CM or CM supplemented with a lower, more optimized concentration (relative to Study 1) of TMAO (5mM).

The equilibrium Young's (E_Y) and dynamic modulus was determined under 10% unconfined compression. Biochemical analysis was performed to determine the GAG, hydroxyproline (OHP) and DNA content. GAG and OHP values were normalized to the DNA content and wet weight. Tested samples were fixed for histology and stained with Picosirius Red and Safranin-O to determine the distribution of collagen and GAG, respectively. Mechanical, biochemical and histological analysis was performed every two weeks (n = 4/5). For study 1, a two-way ANOVA (factors: culture day and treatment) was performed to evaluate the treatment for mechanical and biochemical analysis with a Bonferroni post hoc analysis. For study 2, a Student's t-test was performed to compare properties of the constructs cultured in CM or CM supplemented with TMAO. Significance was set at α=0.05.

Results

By day 28, the mechanical properties of the 55mM and 180mM TMAO groups were 2X greater than the control group (Fig 1). The dynamic modulus followed a similar trend across groups (data not shown). The normalized GAG and collagen content in the 55mM TMAO group were significantly greater than control at day 28 (Fig 2), and histology showed an even distribution of GAG and collagen throughout the thickness of the construct (Fig 3).

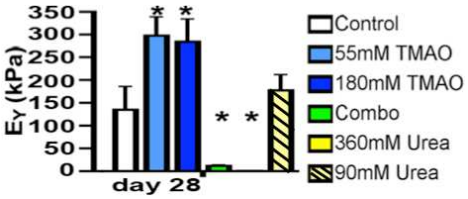

Fig 1: Young's modulus on day 28. * p < 0.05 vs. control.

Lower concentrations of urea did not have adverse effects on the mechanical or biochemical properties (Fig 1 & 2). However, the groups with a high concentration of urea (360mM) did not increase from day 0. While the addition of TMAO with urea helped to slightly mitigate the effects of the urea on the mechanical and biochemical properties, the properties were significantly lower than the control group (Fig 1 & 2).

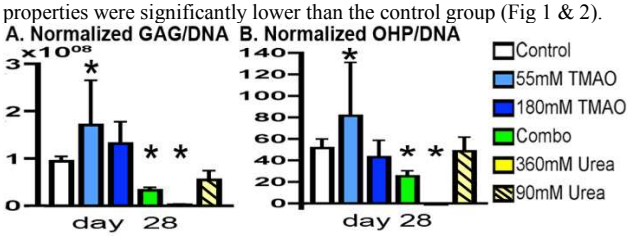

Fig 2: A. Normalized GAG content and B. Normalized OHP content for engineered cartilage constructs of Fig 1. * p < 0.05 vs. control.

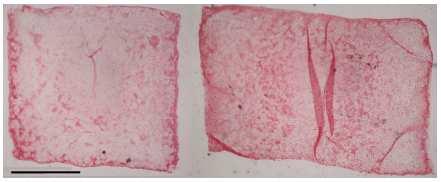

Fig 3: Histological samples at day 28 for control (left) and 55mM TMAO group stained with Picosirius Red for collagen. Bar = 1 mm.

Study 2: Digestion of tissue engineered constructs with cABC decreased the E_Y from 94.3 ± 7.1kPa at day 14 to 15 ± 1.3kPa. CM media supplemented with TMAO exhibited significantly greater mechanical and biochemical properties relative to CM alone by day 28, which persisted to day 42 (Fig 4). By day 42, the E_Y reached 115.6 ± 38.1kPa and 235.6 ± 75.8kPa for CM and TMAO groups, respectively.

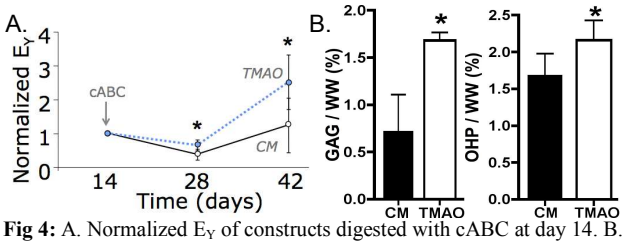

Fig 4: A. Normalized E_Y of constructs digested with cABC at day 14. B. GAG (left) and OHP (right) content normalized to wet weight at day 28 for constructs cultured with CM or CM plus TMAO (TMAO) after day 14 cABC digestion. *p < 0.05 between CM and TMAO.

Discussion

As a culture media supplement, TMAO was observed to significantly increase engineered cartilage mechanical and biochemical properties (Study 1 & 2), leading us to accept our hypothesis. This is the first study, to our knowledge, that has demonstrated the beneficial effects of this organic compound, $(CH_3)_3NO$, on cartilaginous tissue development in culture. TMAO in combination with controlled cABC enzymatic treatment [5] may provide a strategy to increase collagen content towards native cartilage levels, which has remained a significant challenge for the field (Study 2). Future studies aim to optimize the use of TMAO for cartilage tissue engineering as well as to understand the mechanisms that underlie the enhanced tissue properties that are associated with its use.

References

[1] Yancey, PH *J Experimental Biology* 2005; [2] Meersman, F+ Biophysical J 2009; [3] Ng, KW+ *Tissue Eng*, Part A 2010; [4] Lima, EG+ *Osteoarthritis Cartilage* 2008; [5] Bian, L.+ *Tissue Eng*, Part A 2009.

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