

TMAO Supplementation of Culture Media for Engineered Articular Cartilage

Grace D. O'Connell, Neil Dunleavy, Michael Carapezza, Gerard A. Ateshian, Clark T. Hung

Introduction: Sharks have a skeletal structure of cartilage instead of bone. Trimethylamine N-oxide (TMAO: $(\text{CH}_3)_3\text{NO}$) 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]. Previous work in our laboratory observed increased mechanical and biochemical properties of engineered cartilage constructs cultured in media supplemented with TMAO [3]. Inspired by the beneficial impact of TMAO on engineered cartilage, this study aimed to optimize the beneficial effects of TMAO by investigating a range of TMAO concentrations in our culture system. We hypothesize that there will be a dose-dependent relationship between engineered cartilage properties and TMAO concentration.

Methods and Materials: Articular chondrocytes were harvested from juvenile bovine wrist joints and were casted in 2% w/v agarose. Cylindrical constructs (30×10^6 cells/mL, $\phi = 4\text{mm}$, thickness = 2.34mm) were cultured in serum-free chondrogenic media (CM) with 10ng/mL of TGF- β_3 for the first 14 days [4]. The culture media was also supplemented with TMAO at concentrations of 5, 50, 100 and 200 mM during the first 14 days of the 42-day culture period. Control constructs were cultured in CM without TMAO. The Young's modulus and dynamic modulus were calculated at 10% strain in unconfined compression. Mechanical and biochemical analyses (glycosaminoglycan (GAG) and collagen content) were performed every 2 weeks ($n = 4/5$ constructs per time point).

Results and Discussion: The mechanical properties (Young's and dynamic modulus) of constructs cultured with less than 100mM of TMAO were either better than or equal to the control group ($p < 0.05$; Figure 1A). The Young's modulus of the 50mM TMAO group reached native values by day 42 and was $615.4 \pm 8.1\text{kPa}$, which was 13% greater than the control ($p < 0.05$). High concentrations of TMAO (i.e. 200mM) resulted in a decrease in the properties of the engineered constructs. At day 42, the collagen content of the 50mM TMAO group was 27% greater than the control (Figure 1B). The matrix distribution of the TMAO groups was more evenly distributed than the control group (Figure 2 – shown for 50mM TMAO sample).

Conclusions: As a culture media supplement, TMAO was observed to significantly increase engineered cartilage mechanical and biochemical properties. Contrary to our initial hypothesis, the relationship between the improved properties and the TMAO concentration was not dose-dependent. Interestingly, the GAG content did not differ between the control and the experimental groups (Figure 1C), which may suggest that the TMAO compound affects collagen production, including perhaps the protein folding. The similar increases in collagen content and dynamic modulus with TMAO concentration may suggest a relationship between collagen and the dynamic modulus. These findings support the role of TMAO supplementation as a strategy to increase collagen content of engineered cartilage, a significant challenge that remains for the field. Future studies aim to understand the mechanisms that underlie the enhanced tissue properties that are associated with TMAO in hopes of optimizing its use for cartilage tissue engineering.

Acknowledgements: This work was supported in part by NIAMS grants AR52871, AR46568 and a diversity supplement.

References: [1] Yancey+ *J Exp Biol*, 2005: 208, 2819-30; [2] Meersman+ *Biophys J*, 2009: 97,2559-66; [3] O'Connell+ *Proceedings of ORS*, 2011; [4] Ng+ *Osteoarthritis Cartilage*, 2009: 17, 220-7.

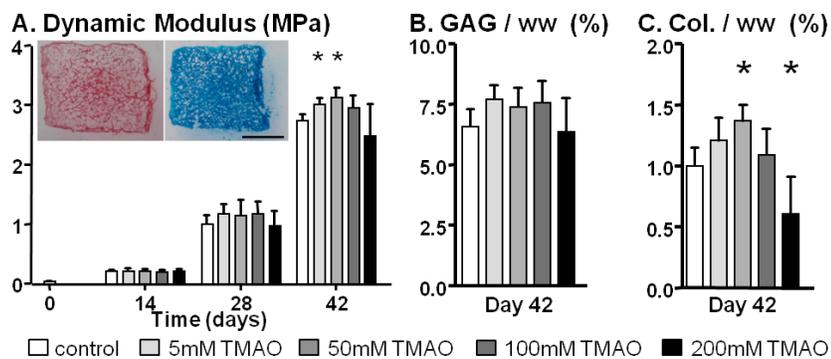


Figure 1: A) Dynamic modulus, B) collagen content, and C) GAG content normalized by wet weight (ww). * represents a significant difference with control. Inset: Histological staining of a representative sample from the 50mM TMAO group at day 42; collagen (left) and GAG (right). Bar = 1mm.