# Delivery of N-Acetylcysteine via Electrospun Scaffolds to Regulate Glutathione and Transsulfuration Pathways

Kubra Yigit[1], Anthony Callanan[1]

k.yigit@sms.ed.ac.uk

[1]Institute for Bioengineering, School of Engineering, University of Edinburgh

Full paper*

## Introduction

Osteoarthritis (OA) is a prevalent degenerative joint condition that affected an estimated 0.6 billion people worldwide in 2021 [1]. There is growing recognition that reactive oxygen species (ROS) significantly contribute to OA pathogenesis by promoting oxidative damage in cartilage [2]. In this context, antioxidant-based strategies have emerged as a promising approach to mitigate oxidative stress and restore redox balance in cartilage tissue. N-acetylcysteine (NAC) serves as an effective antioxidant by reducing ROS levels and supporting intracellular redox balance via glutathione production and the transsulfuration pathway [3]. In this study, NAC-loaded electrospun scaffolds were developed for targeted antioxidant delivery in cartilage, and their effects on intracellular redox pathways, chondrocyte phenotype retention, and ROS neutralization were investigated.

## Methodology

### Scaffold Fabrication

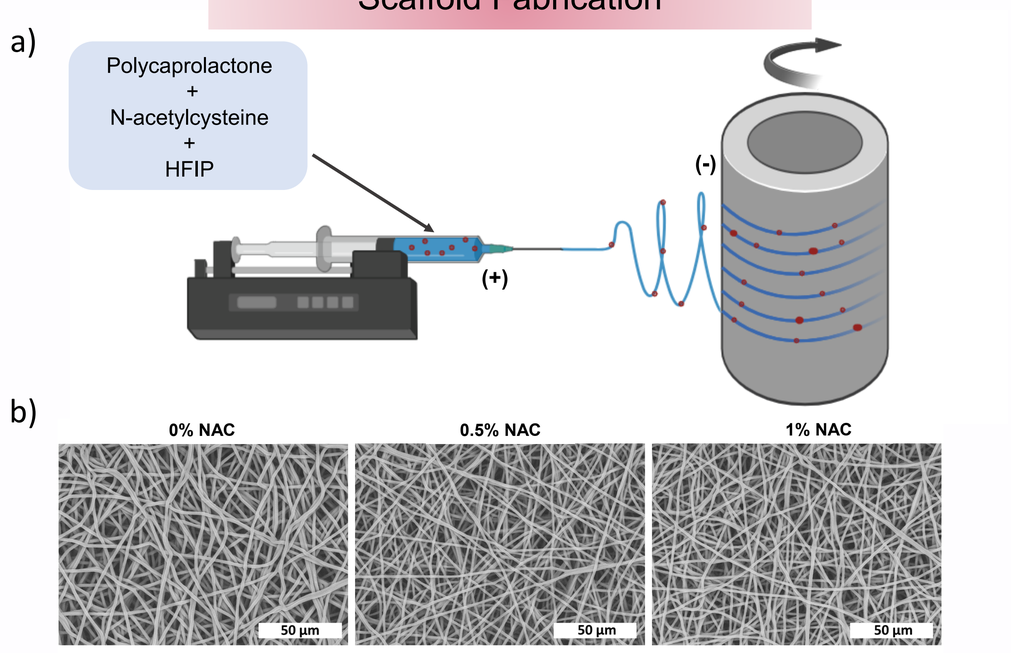



**Figure 1.** (a) Schematic illustration of the electrospinning process used for scaffold fabrication. Created with BioRender.com (b) Representative morphology of the resulting fibrous scaffolds (Scale bar = 50 µm). (HFIP= 1,1,1,3,3,3-Hexafluoro-2-propanol)

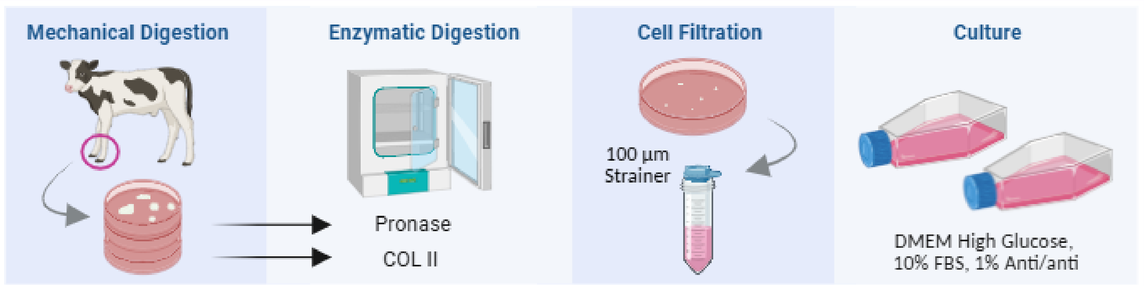



**Figure 2.** Isolation and culture of primary chondrocytes from bovine cartilage tissue. Created with BioRender.com

## Scaffold Characterization

### Mechanical Testing

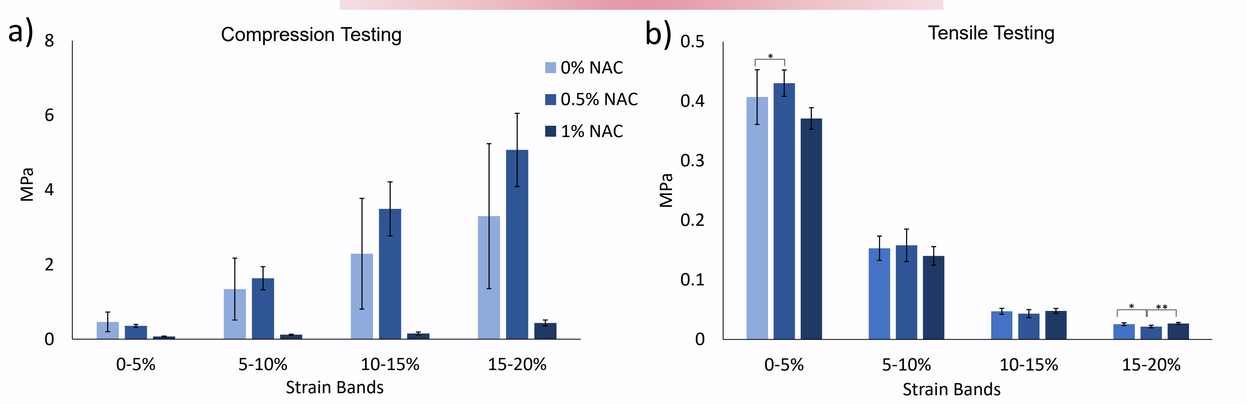



**Figure 3.** (a) Incremetal compressive moduli of scaffolds. (b) Incremental tensile moduli of scaffolds. Data represent mean ± SD (n = 4). One-way ANOVA with Tukey's post hoc test; * p < 0.05, ** p < 0.01.

### Antioxidant Activity

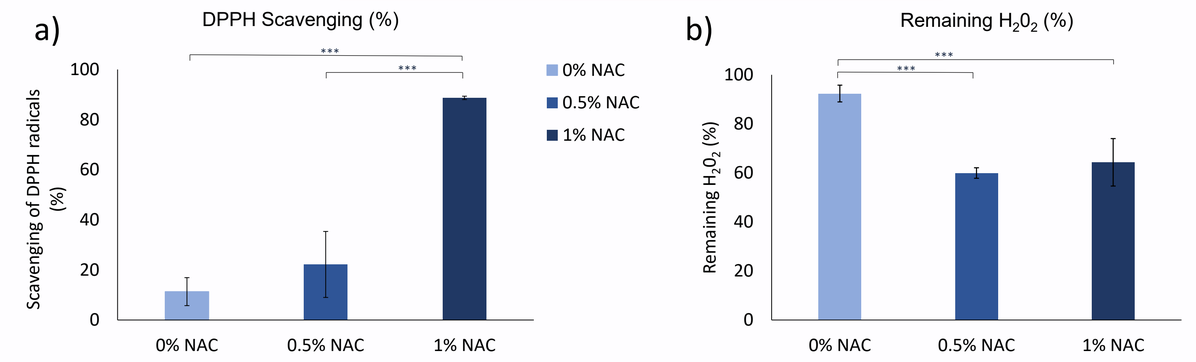



**Figure 4.** (a) DPPH radical scavenging ability (%) of scaffolds and (b) percentage of remaining $H_2O_2$ after 24 h. Data represent mean ± SD (n = 5). One-way ANOVA with Tukey's post hoc test; *** p < 0.001.

## Cellular Response

### Cell Viability, DNA Content and Staining

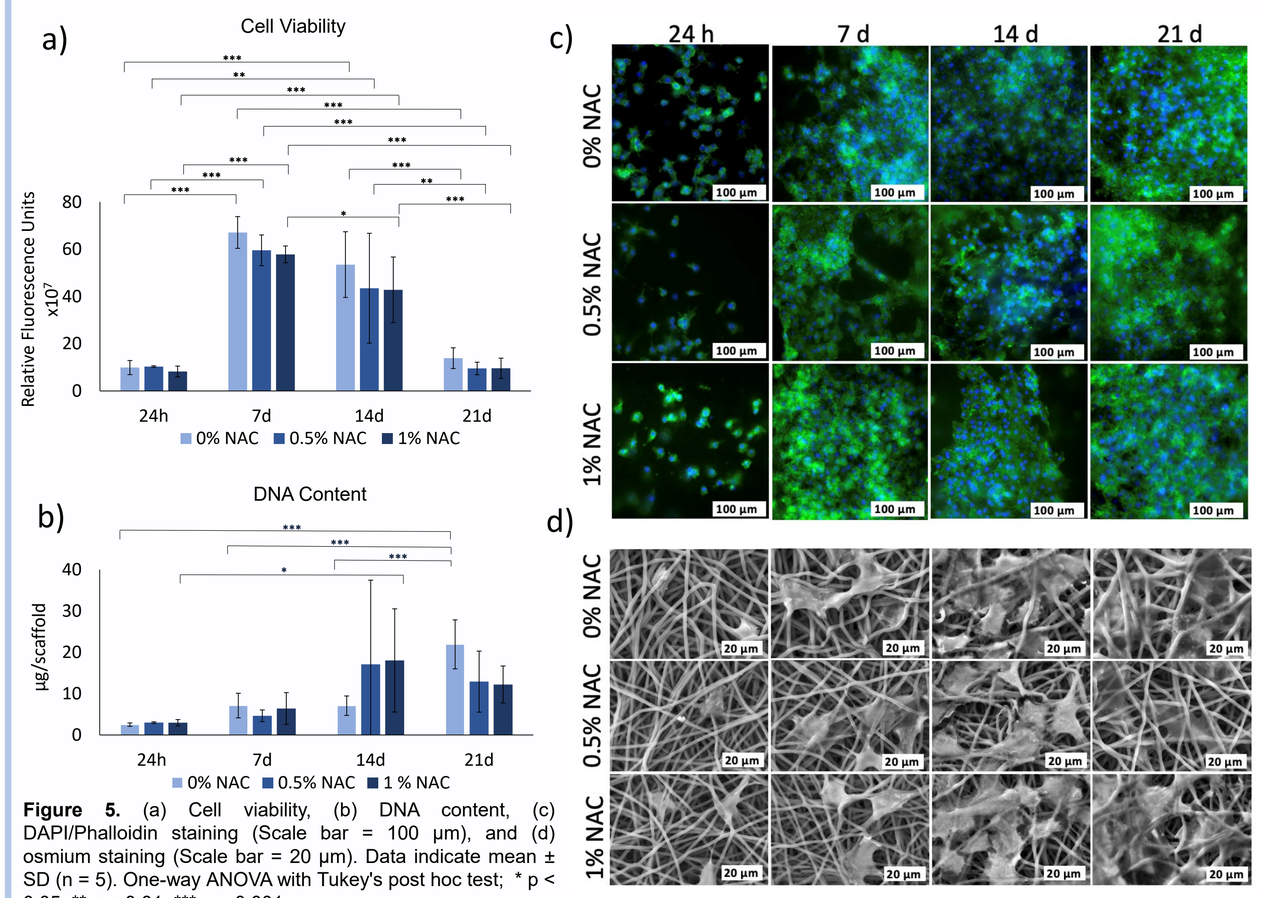



**Figure 5.** (a) Cell viability, (b) DNA content, (c) DAPI/Phalloidin staining (Scale bar = 100 µm), and (d) osmium staining (Scale bar = 20 µm). Data indicate mean ± SD (n = 5). One-way ANOVA with Tukey's post hoc test; * p < 0.05, ** p < 0.01, *** p < 0.001.

### Cartilage Gene Expression

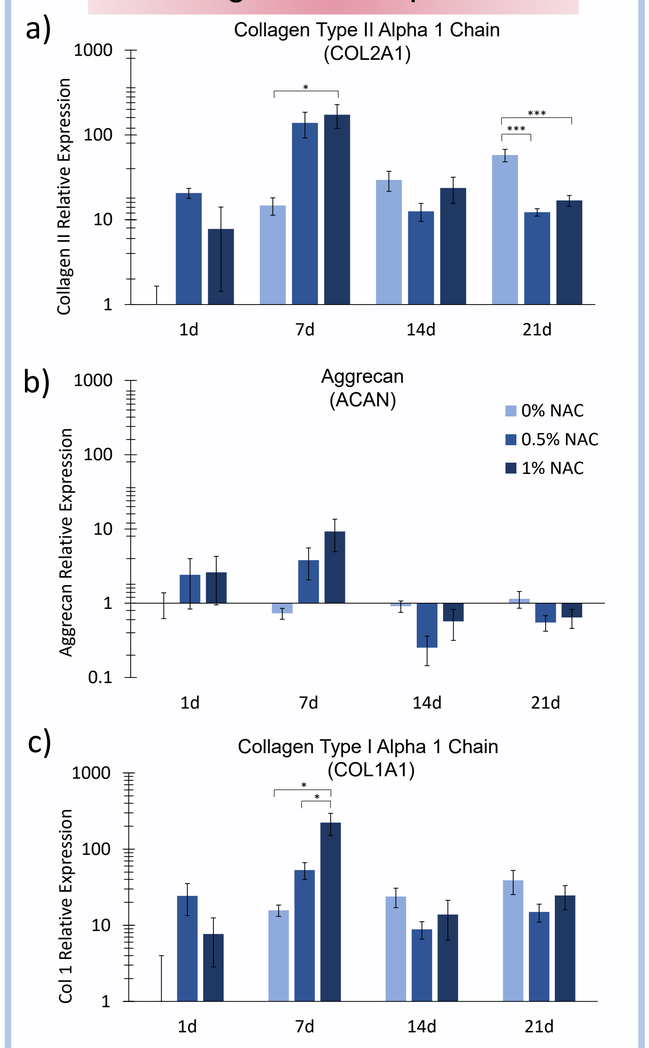



**Figure 6.** Relative gene expression of (a) COL2A1, (b) ACAN, and (c) COL1A1 normalized to 24 h 0% NAC. Error bars represent SEM (n = 5). One-way ANOVA with Tukey's post hoc test; * p < 0.05, *** p <0.001.

### Glutathione Gene Expression

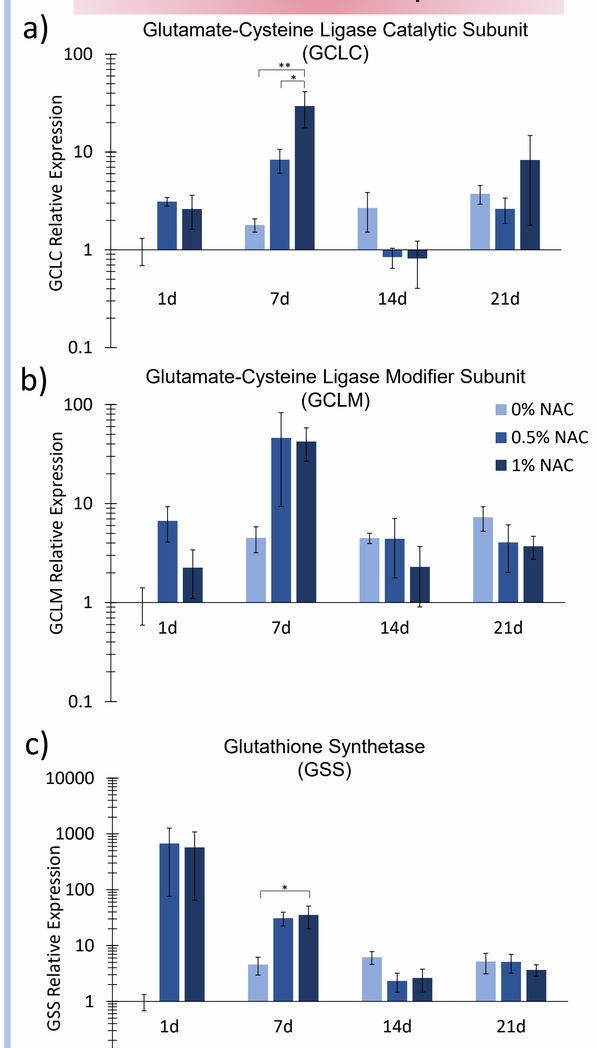



**Figure 7.** Relative expression of (a) GCLC, (b) GCLM, and (c) GSS. Error bars show SEM (n = 5). One-way ANOVA with Tukey's post hoc test; * p < 0.05, ** p < 0.01.

### Transsulfuration Gene Expression

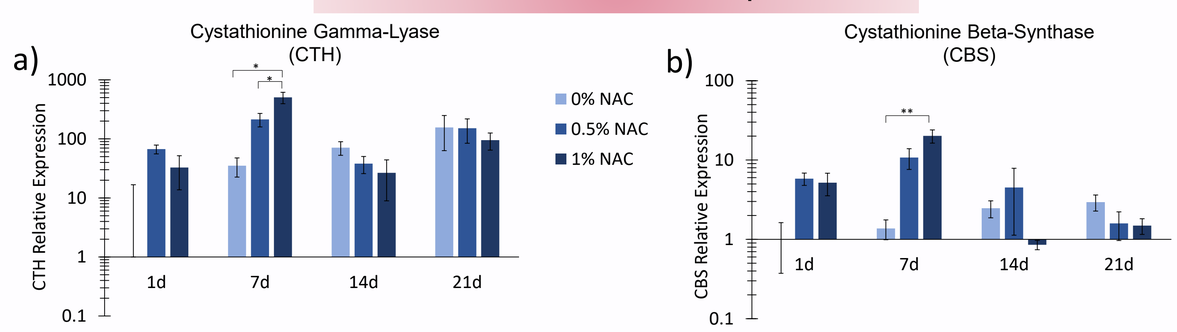



**Figure 8.** Relative gene expression of (a) CTH and (b) CBS normalized to 24 h 0% NAC. Error bars show SEM (n = 5). One-way ANOVA with Tukey's post hoc test; * p < 0.05, ** p < 0.01.

## References

1. Qiao et al., Arthritis Research & Therapy 2025.
2. Riegger et al., Cellular & Molecular Biology Letters 2023. 3. Yigit et al., Macromolecular Materials and Engineering 2026*.

## Acknowledgements

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## Conclusion

The findings indicate that incorporating NAC improved the antioxidant performance of the scaffolds in the extracellular environment, while also modulating cellular responses in chondrocytes. Specifically, scaffolds containing 1% NAC influenced the expression of genes involved in the glutathione (GCLC, GCLM, GSS) and transsulfuration (CBS, CTH) pathways, underscoring their role in maintaining redox homeostasis. This regulation may contribute to the preservation of the chondrocyte phenotype through redox-sensitive mechanisms. The ability to modulate oxidative stress both outside and within the cells highlights the advantage of integrating NAC delivery into scaffold design. Such an approach may enhance the long-term stability and functionality of the scaffolds, offering a promising strategy for improving cartilage repair outcomes.