In vitro Evaluation of a Hydrolyzed Collagen on Fibroblasts and Macrophages

Fahimeh Tabatabaei[1], Tiffany Fawn Sanchez[1], Sergio Medina[1], Nina Bionda[1]
[1]Rochal Technologies, R&D division of Sanara MedTech, San Antonio, TX
Email of Presenting Author: FTabatabaei@sanaramedtech.com

Disclosures: Fahimeh Tabatabaei (Rochal Technologies), Tiffany Fawn Sanchez (Rochal Technologies), Sergio Medina (Rochal Technologies), Nina Bionda (Rochal Technologies)

INTRODUCTION: Fibroblasts and macrophages play an important role in tissue repair following surgery, which orchestrates inflammation, matrix remodeling, and tissue regeneration. Hydrolyzed Type I collagen, composed of low molecular weight peptides known as matrikines, may influence the surgical wound environment and cellular responses. While hydrolyzed collagen is used clinically, its effects on fibroblast migration and macrophage phenotypes are not well characterized. This study aimed to evaluate the effects of an ultra-low molecular weight Type I bovine hydrolyzed collagen (<3 kDa), on fibroblast migration and proliferation and on macrophage behavior in a controlled *in vitro* setting.

METHODS: Human primary dermal fibroblasts (ATCC PCS-201-012) and murine RAW 264.7 macrophages (M0 and LPS-activated M1) were treated with varying concentrations of hydrolyzed collagen. Fibroblast migration was assessed via a scratch-wound assay in 48-well plates, and wound closure was quantified after 6 hours. Proliferation was evaluated via short-term (2-minute exposure with 24-hour incubation) and long-term (24- and 48-hour continuous exposure) assays using AlamarBlue and MTT methods, respectively. Macrophage proliferation was quantified via PicoGreen, gene expression via qPCR, and cytokine production via Luminex multiplex analysis. All experiments were performed in triplicate and repeated independently. Statistical significance was determined using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

RESULTS: In fibroblasts, *in vitro* exposure to hydrolyzed collagen increased cell migration at 0.03 mg/mL and 0.3 mg/mL compared to control ($p < 0.05$). Short-term exposure (2 minutes) to higher concentrations (30–300 mg/mL) enhanced fibroblast metabolic activity after 24 hours without cytotoxicity. Longer-term exposure showed a concentration-dependent increase in proliferation, with the highest observed value at 30 mg/mL after 48 hours (192.5% ± 13.8% of control, $p < 0.05$) (Figure 1). In macrophages, hydrolyzed collagen increased M0 proliferation while maintaining M1 viability. Gene expression analysis revealed upregulation of M2-associated markers (IL-10, VEGF-A, MMP9, CCL2) in M0 cells and increased IL-10 expression in M1 cells. Cytokine profiling indicated elevations in VEGF, CCL22, and IL-10 without upregulation of TNFα or IL-1β in M1 macrophages (Figure 2).

DISCUSSION: These *in vitro* findings demonstrate that hydrolyzed collagen promotes fibroblast migration and proliferation and modulates macrophage behavior in a context-dependent manner. The biphasic fibroblast response and macrophage phenotypic trends suggest that hydrolyzed collagen fragments may influence cellular responses relevant to tissue repair. While these results are limited to controlled *in vitro* models, they provide mechanistic insight into potential cellular interactions in the surgical wound environment.

SIGNIFICANCE/CLINICAL RELEVANCE: Hydrolyzed collagen provides a collagen-based environment that supports fibroblast and macrophage responses *in vitro*. These findings suggest a potential role for hydrolyzed collagen in modulating the cellular environment relevant to surgical site management, warranting further investigation.

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IMAGES AND TABLES:

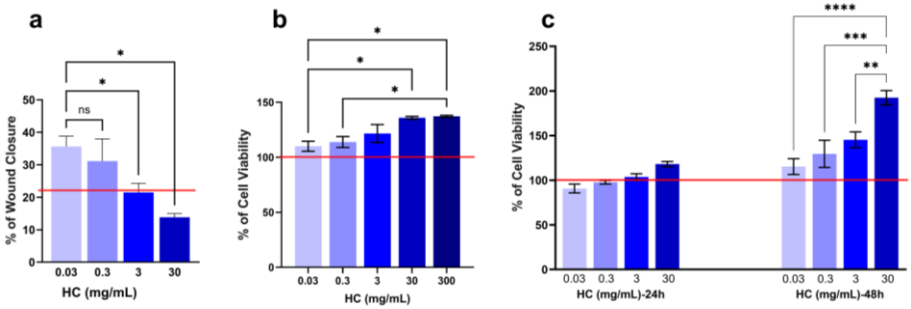

Figure 1: *Fibroblast response to hydrolyzed collagen (HC) treatment. (a) Migration (% wound closure) after 6 hours assessed by scratch-wound assay. (b) Metabolic activity (% of control) after 2-minute exposure, measured at 24 hours. (c) Proliferation (% of control) after 24 and 48 hours of continuous exposure. Data represent mean ± SEM. *p < 0.05 vs. untreated control.*

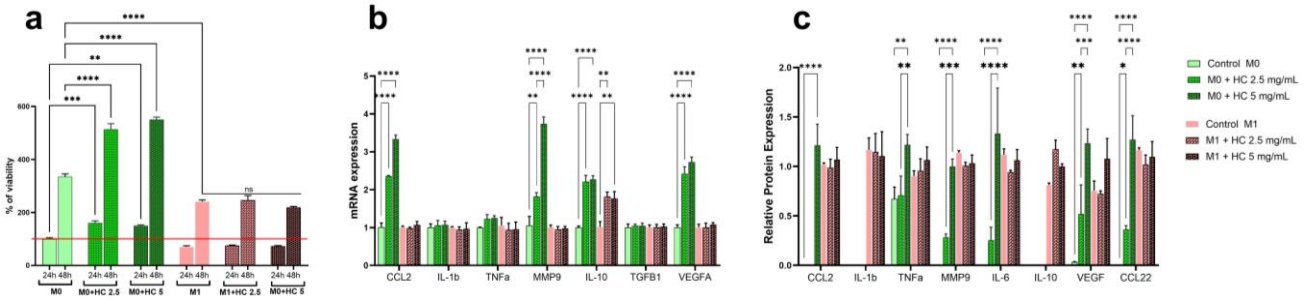

Figure 2: *Macrophages response to hydrolyzed collagen (HC) treatment. (a) Proliferation of M0 and M1 macrophages treated with CellerateRX (2.5 and 5 mg/mL) at 24 and 48 hours. (b) Gene expression analysis of M0 and M1 macrophages at 48 hours. (c) Cytokine and protein expression profiles from cell lysates at 48 hours. Data represent mean ± SEM, *: p < 0.05 vs. control.*