Collagen scaffold with gallic acid-assisted crosslinking: potential application for bone tissue engineering

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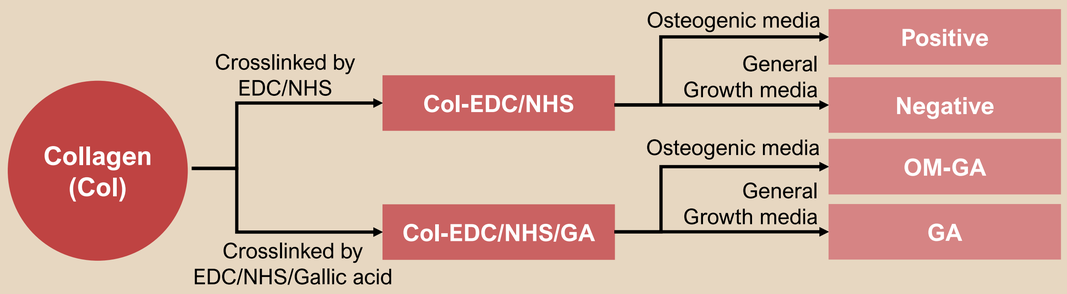
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Introduction

The increasing global demand for orthopedic implants, driven by trauma and aging populations, highlights a critical need for bioactive alternatives to traditional metal implants, which cannot restore vital biological functions like mineral regulation. Bone tissue engineering provides a customizable, scalable solution to overcome the finite supply and immunogenicity risks associated with autografts and allografts. Human adipose-derived stem cells (hADSCs) are an ideal progenitor for these applications due to their high-volume yield and minimally invasive harvest. However, hADSCs exhibit an adipogenic bias, naturally favoring fat formation over bone. While collagen is a common biomimetic material, its clinical utility is often restricted by rapid enzymatic degradation. Gallic acid (GA) is a non-toxic polyphenol that serves as a versatile crosslinker capable of supporting bone formation while suppressing adipogenesis. This study employs a novel liquid-phase crosslinking method with EDC/NHS/GA GA to enhance the stability of the collagen matrix and direct stem cell fate. The goal is to evaluate ability of gallic acid-incorporated collagen scaffolds to suppress adipogenic regulators and maintain osteogenic signaling over 28 days for functional bone repair.

Methods

Collagen was extracted from porcine skin using pepsin digestion and a double salt-precipitation process. These scaffolds were produced using a novel liquid-phase crosslinking method at 4◦C with an EDC/NHS cocktail (Col-EDC/NHS), where the experimental group (Col-EDC/NHS/GA) incorporated 50 mM of gallic acid (GA). Post-synthesis, the scaffolds were lyophilized and sterilized via 15 kGy gamma irradiation. Chemical integration was verified using ATR-FTIR and ssNMR spectroscopy, while physical properties were assessed through SEM for microarchitecture and DSC for thermal behavior. Material stability was evaluated through a 28-day biodegradation test in PBS and daily GA release quantification using a Fast Blue BB assay. Initial cell-material interactions were established using L929 murine fibroblasts. Metabolism and cytotoxicity was respectively tested using PrestoBlue® and Live/Dead assays, while adhesion and proliferation were quantified via PicoGreen™ and SEM at 24 and 72 hrs.
hADSCs were cultivated on Col-EDC/NHS and Col-EDC/NHS/GA (GA), cultivated in either general growth media or osteogenic media (OM) for 28 day. Differentiation was tracked using qRT-PCR targeting specific markers for osteogenesis (*RUNX2, COL1A1, SPP1*), chondrogenesis (*SOX9*), and adipogenesis (PPARɣ) on day 14 and 28. Histological analysis, targeted on potentially trilineage differentiation, performed on day 28. Alkaline Phosphatase activity was measured on day 14 to support qRT-PCR and histology results.



Discussion and Conclusion

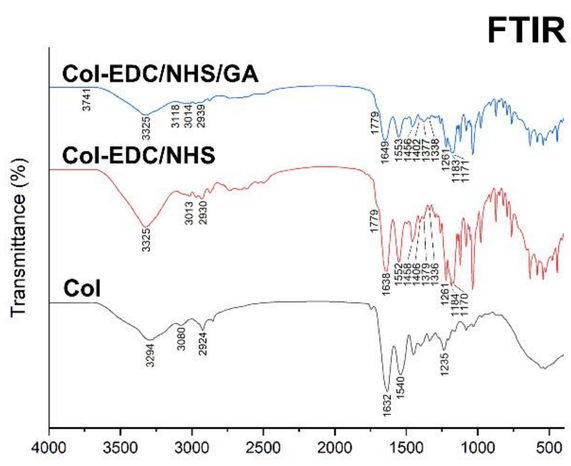
The research establishes that a unique liquid-phase crosslinking technique utilizing EDC, NHS, and Gallic Acid converts collagen into an osteomodulative environment that effectively overcomes the biological hurdles associated with hADSCs. Although hADSCs are highly valued for their ease of harvest and rapid proliferation, their use in regenerating bone is often complicated by an innate tendency to develop into adipocytes. This investigation shows that the chemical bonding of GA creates a durable foundation that redirects stem cell development away from this fatty lineage. Col-EDC/NHS/GA scaffolds demonstrate excellent physical qualities by remaining intact during degradation test. This is a significant improvement, as Col-EDC/NHS could not withstand the same conditions. These enhanced scaffolds successfully facilitate cell attachment and adhered on the structure while providing acceptable cytotoxicity niche in tissue culture. By simultaneously inhibiting the adipogenic supporter and boosting bone-specific markers, the Col-EDC/NHS/GA system ensures that hADSCs prioritize bone development. This occurs even when the cells are exposed to stimuli that would normally promote adipogenesis. Following a 28-day growth period, the hADSCs reached a functional bone-forming state characterized by secreting collagen type I and calcium minerals. This successful transition proves that GA-modified collagen scaffolds represent a superior material for bone repair, offering a reliable method to bypass the natural developmental limitations of adipose-derived cells.

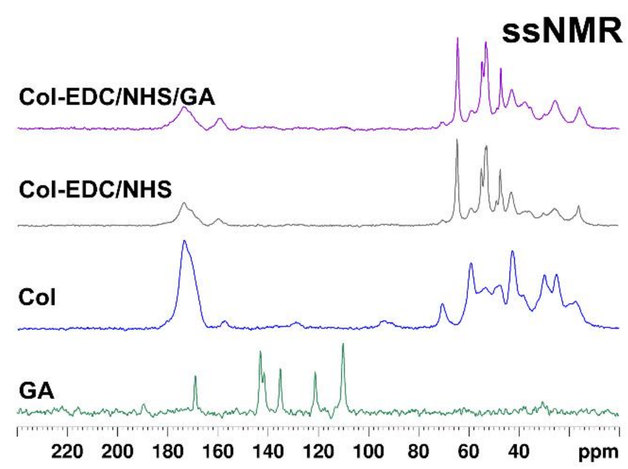
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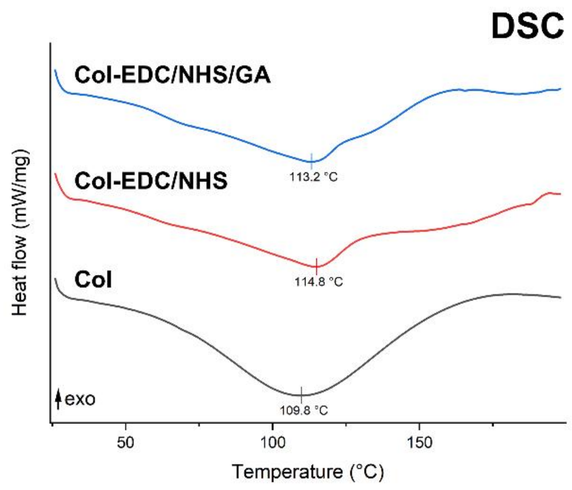
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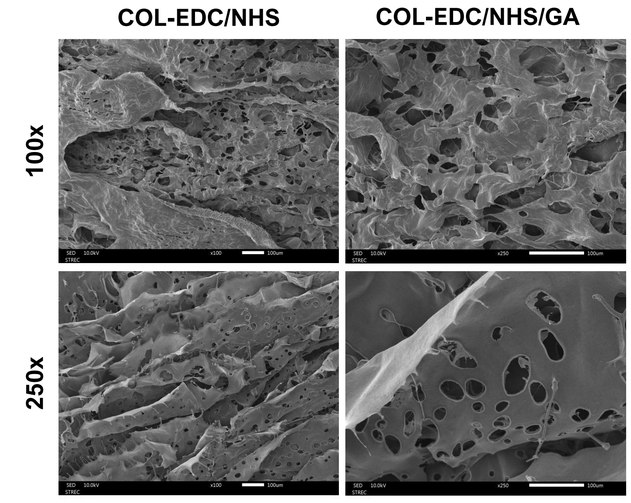
Results

Material Characterization

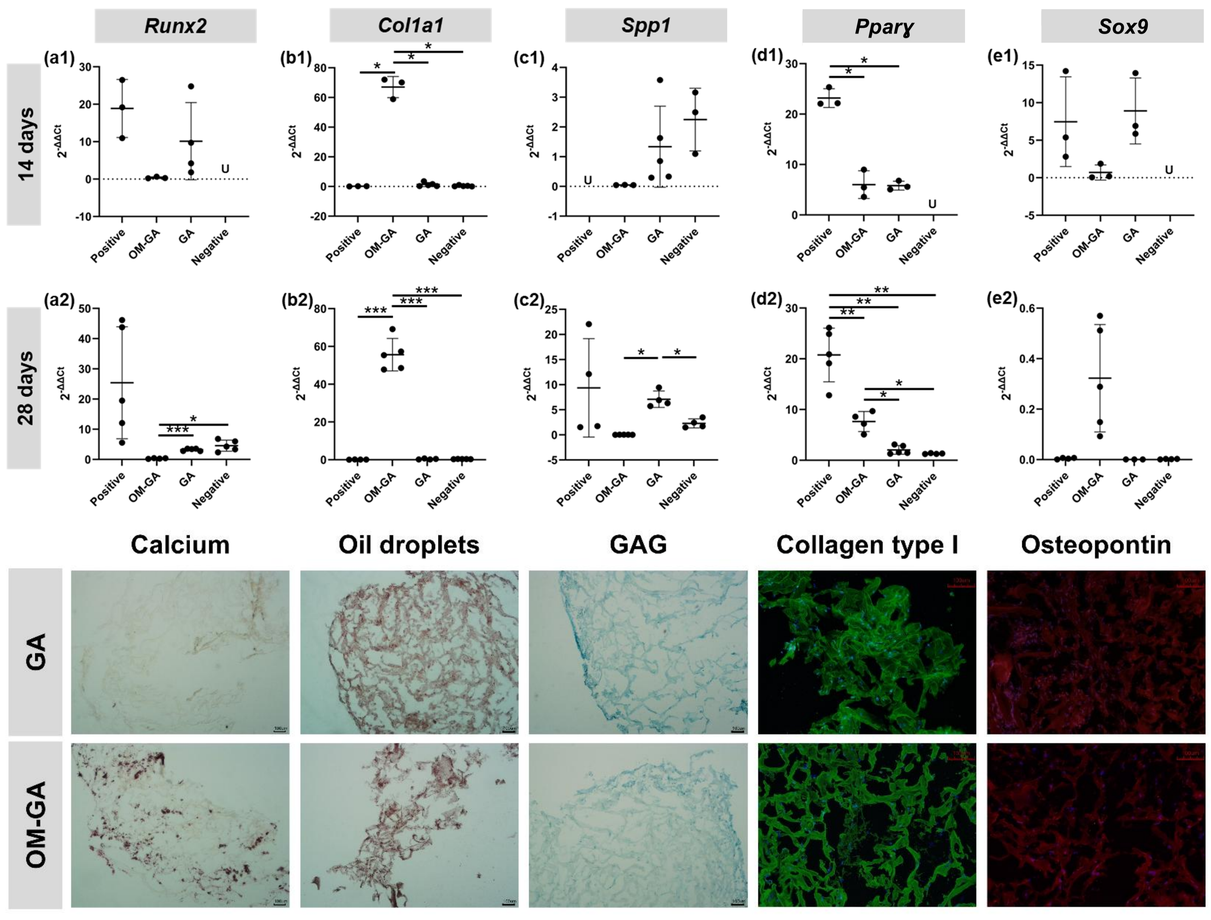








qRT-PCR & Histological analysis



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