Biodegradable, Fibrous Gelatin Scaffolds with Tunable Piezoelectricity Promote Cartilage Formation under Mechanical Loading Conditions

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INTRODUCTION: Piezoelectric, smart materials have gained interest as scaffolds for cartilage repair applications due to their self-powered capability for electrical stimulation when subjected to mechanical loading. However, limitations exist with currently explored biomaterials, which are either non-degradable [1] or may have long-term biocompatibility issues due to degradation products [2]. In this study, we processed gelatin into fibrous scaffolds followed by electric poling to tailor piezoelectric properties. We investigated this scaffold for promoting the chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells (MSCs) under dynamic compression as a potential scaffold to treat cartilage defects.

METHODS: Gelatin (bovine skin) were electrospun using standard conditions to form random fibrous mats and crosslinked, according to our previously reported protocols [3]. The scaffolds were poled by exposure to a high electric field to render the scaffolds piezoelectric [4]. The following scaffolds were studied: negatively poled (Poled(–)) and positively poled (Poled(+)), which the + and – signs are based on the direction of the applied electric field, and the unpoled scaffold, which serves as a control. The piezoelectric properties of scaffolds were tuned by poling using different electric fields. Voltage output was measured using cyclic compression at 10% deformation using a custom sensor (n=3 per group). Mechanical testing (compression and tension) was performed to determine Young's modulus (n=5 per group). MSCs (male and female donors) were cultured onto Poled(–), Poled(+) and unpoled gelatin in chondrogenic induction media in static culture conditions over 28 days. For mechanical loading, unpoled and poled gelatin scaffolds loaded with MSCs were placed in bioreactors and subjected to continuous media perfusion for 28 days. Dynamic compression was applied from days 14 to 28 at 1 Hz frequency with 10% deformation for 3 h (1 h on and 1 h off) per day for up to 28 days. Cell growth was evaluated using the PicoGreen DNA assay. Production of sulfated glycosaminoglycan (GAG) was measured by the DMMB assay. Production of collagen types I and II and aggrecan was determined by ELISA and immunostaining by confocal imaging. For histology, samples were fixed and processed using paraffin-embedding histology. Cross-sections were stained with hematoxylin and eosin (H&E) and safranin O for proteoglycans. N=4 per group were studied for all quantitative biochemical assays. Chondrogenic and hypertrophic gene expressions were studied using qRT-PCR (n=3 per group). Statistical analysis was performed using one-way ANOVA with comparisons by Tukey's posthoc test ($p<0.05$).

RESULTS: Varying the degree of electric poling for gelatin scaffolds achieved tunable piezoelectric properties (Fig. 1D-F). A clear voltage output with the peak voltage of 47 and 95 mV for low piezo (LP) and high piezo (HP) poled groups were measured, while a consistent waveform was not detected for the unpoled group (Figure 1D,E). The corresponding piezoelectric coefficients, d_{33}, for poled groups were also measured (Figure 1F). Poling did not alter mechanical properties of gelatin scaffolds (Figure 1G). LP scaffolds were used for subsequent cell studies. All constructs harvested from bioreactors exhibited a smooth and shiny surface characteristic of hyaline cartilage as shown in Figure 2A. Histological staining of scaffolds showed rounded and lacuna-embedded chondrocyte morphology for all groups, while poled undergoing dynamic compression mimicked cell organization of native cartilage with flattened to elliptical cell morphology in the superficial layer transitioning to rounder cell shapes in the middle layer with an intense and uniform proteoglycan staining (Figure 2B). Intense immunofluorescent staining for both collagen type II and aggrecan were also detected for poled gelatin under dynamic compression conditions (Figure 2C). All scaffold groups had statistically similar cell numbers at day 28 (Figure 2D). The poled gelatin scaffold with dynamic compression group had the highest sulfated GAGs per cell and collagen type II with the lowest collagen type I production (Figure 2E-G). Interestingly, the gene expression for hypertrophic markers, RUNX2, COLX, VEGF-A and COL1, decreased for Poled(–) relative to Poled(+) and unpoled scaffolds in static conditions (Fig. 2H). Ongoing studies are evaluating gene expression under dynamic loading conditions.

DISCUSSION: Our findings establish, for the first time, that random fibrous gelatin can exhibit tunable piezoelectricity when subjected to physiological loading. The piezoelectric, poled gelatin scaffolds showed promise in promoting chondrogenic differentiation and cartilage matrix formation under dynamic load, while mechanical stimulation alone did not enhance chondrogenesis, suggesting a synergistic effect of electromechanical stimulus on stem cell differentiation. Ongoing studies are investigating the underlying mechanism of piezoelectric stimulation using the gelatin scaffolds. Futures studies will evaluate piezoelectric gelatin in an *in vivo* model.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates the potential of degradable piezoelectric gelatin scaffolds as an electromechanical stimulator for cartilage repair.

REFERENCES: [1] Damaraju, et al, Biomaterials, 2017, [2] Rajabi, et al, Acta Biomaterialia, 2015, [3] Huang, et al, Tissue Eng-Part A, 2017, [4] Arinzeh, et al, US Patent, 2023.

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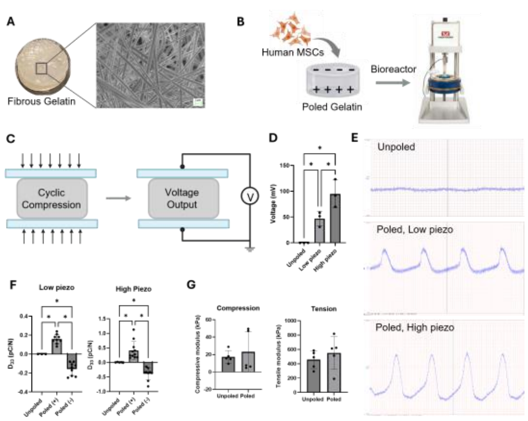

*Figure 1. (A) Scanning electron microscopy of fibrous gelatin, (B) Schematic of MSCs culture on gelatin scaffolds that were subjected to electric poling (Poled) to render them piezoelectric and the compression bioreactor. (C) Schematic of electric output of piezoelectric gelatin scaffold under compression. (D, E) Voltage output of scaffolds that were unpoled (non-piezoelectric) and poled using low (Low Piezo) and high (High Piezo) electric fields, (F) Piezoelectric coefficient d_{33} based on direction of applied electric field (+ or -) (G) Characterization of mechanical properties: Elastic modulus under compression and tensile tests. (*p<0.05)*

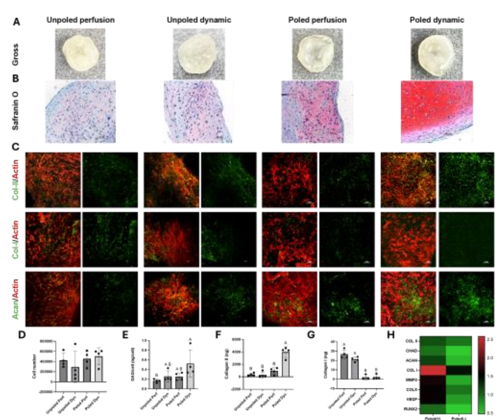

Figure 2. Representative images of unpoled and poled gelatin scaffolds after 28 days undergoing chondrogenesis in perfusion and dynamic bioreactors. (A) Gross images, scale bar 6 mm, (B) Histological images stained with Safranin O. (C) Immunostaining for Collagen II, Collagen I and Aggrecan. Scale bar 100 µm. (D) Cell growth, Quantitative measure of matrix production: (E) GAG (F) Collagen II (G) Collagen I. A is significantly different from B (p<0.05). (H) Gene expression in static culture – fold change compared to unpoled.