

Supplementary Information

Anisotropic mineralized collagen scaffolds accelerate osteogenic response in a glycosaminoglycan-dependent fashion

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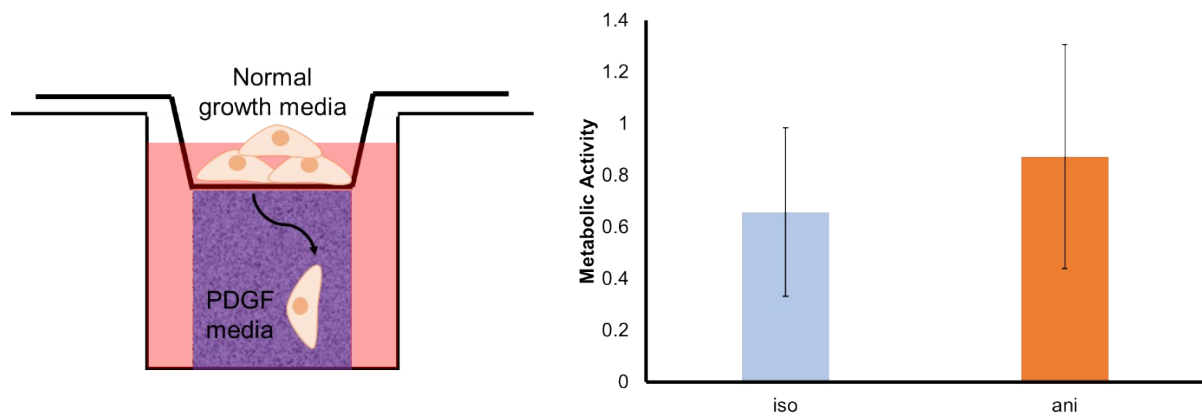
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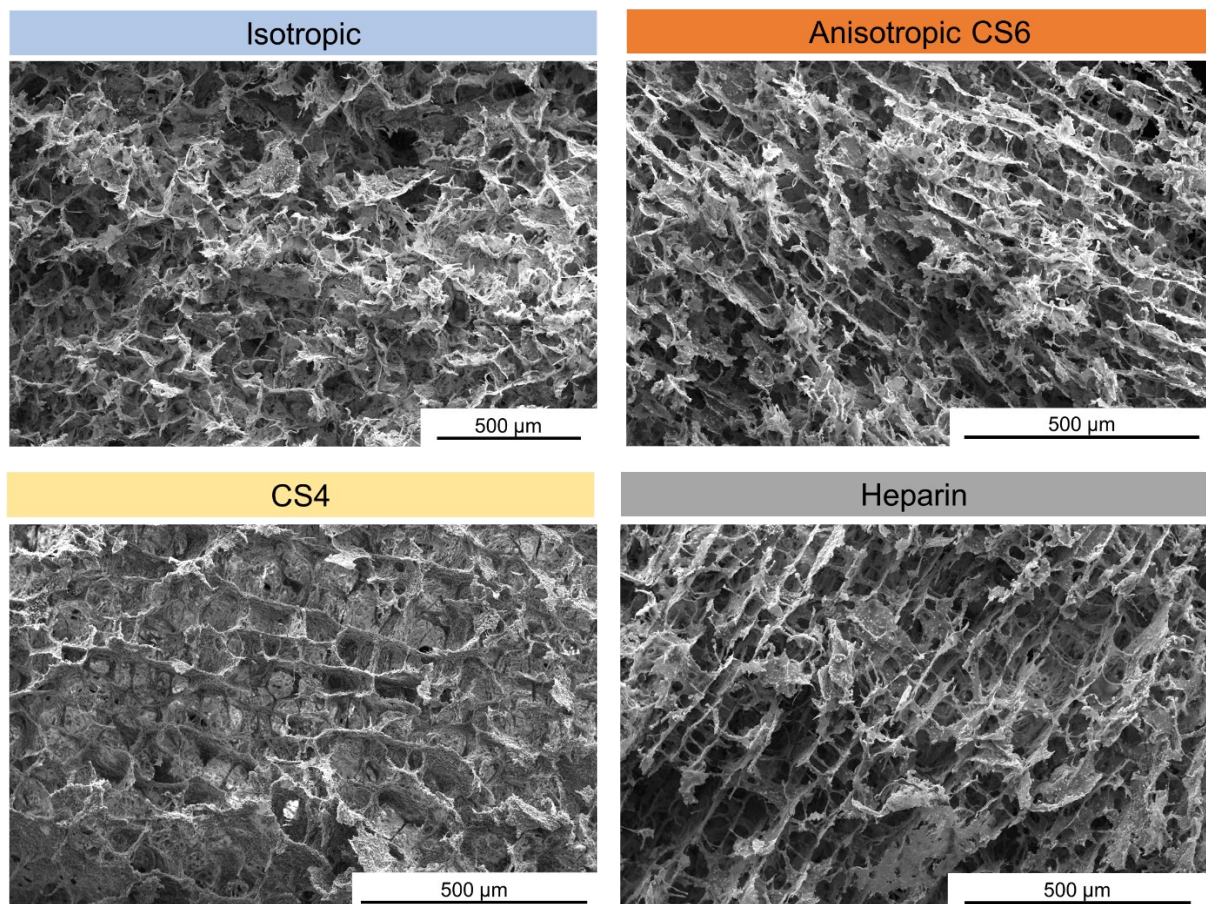
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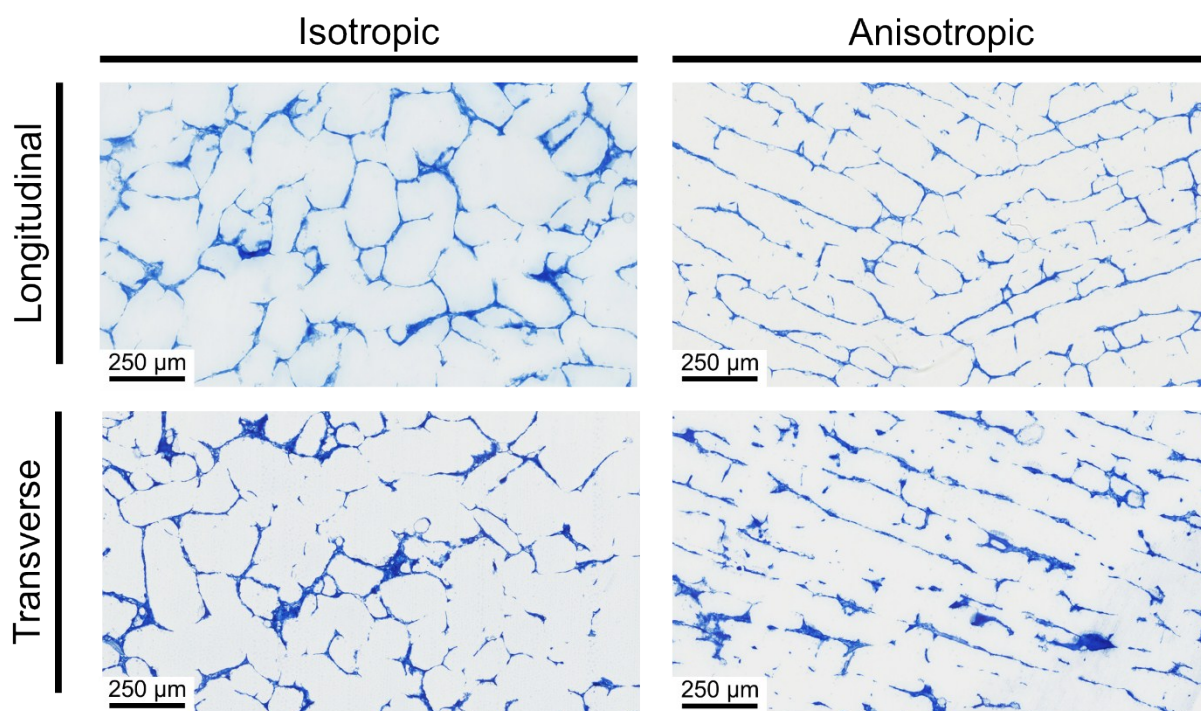
Supplementary Figures



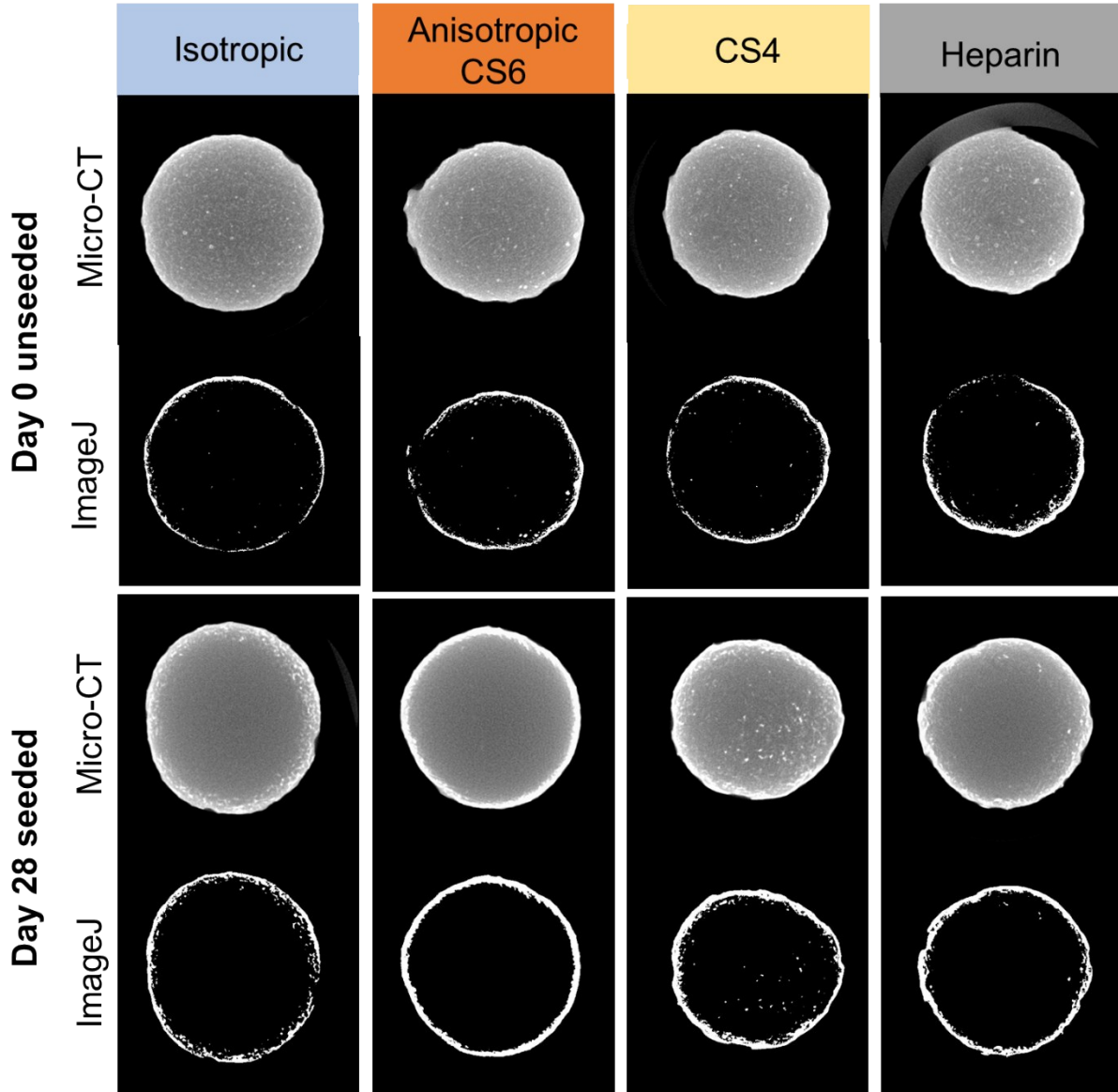
Supplementary Figure 1: Representative image of transwell-seeded scaffolds and the metabolic activity of isotropic and anisotropic scaffold after 24-hours. Anisotropic and isotropic scaffolds containing chondroitin-6-sulfate were placed below transwells in media containing PDGF growth factor and 50,000 hMSCs were seeded on transwell membrane above scaffolds. Cells were allowed to migrate into scaffolds for 24 hours. After 24 hours, scaffolds were removed and the metabolic activity of scaffolds was measured with an alamar blue assay. A value of 1 represents the metabolic activity of 50,000 cells seeded on the transwells as the start of the experiment. Data expressed as average \pm standard deviation (n=6).



Supplementary Figure 2. SEM of pore structure of isotropic chondroitin-6-sulfate (CS6), anisotropic chondroitin-6-sulfate (CS6), anisotropic chondroitin-4-sulfate (CS4), and anisotropic heparin sulfate (Heparin) scaffolds. Isotropic scaffolds demonstrate random round-shaped pore structure and anisotropic scaffolds demonstrate square, elongated pores regardless of glycosaminoglycan content.



Supplementary Figure 3. Representative aniline blue stained sections of isotropic and anisotropic mineralized collagen scaffolds with glycosaminoglycan chondroitin-6-sulfate.



Supplementary Figure 4. Representative Micro-CT scans and their ImageJ threshold equivalents. Chondroitin-6-sulfate, chondroitin-4-sulfate, and heparin sulfate-containing scaffolds are represented by CS6, CS4, and H (respectively). The scaffolds seeded with hMSCs for 28 days are compared to unseeded scaffolds after the hydration process. Quantitative graphs were produced by comparing the scaffold area with the ImageJ white areas in a stack of Micro-CT images.

Supplementary Tables

Supplementary Table 1. Parameters for microwave assisted acid digestion of samples.

Digestion Parameters	Values
Power	1800 W
Temperature	200 °C
Ramp Time	30 minutes
Hold Time	20 minutes

Supplementary Table 2. Parameters for analysis of calcium and phosphorus via Optima 8300 ICP-OES.

ICP-OES Parameters	Values
RF Power	1500 Watts
Nebulizer	GemCone Low Flow
Nebulizer Gas Flow rate	0.85L/min
Plasma Gas Flow rate- Argon	10L/min
Sample Flow rate	1.50mL/min

The following emission lines were used for the quantification of Ca and P
Ca (II)-319.93 nm
P (I)- 213.62 nm