COMPOSITE BIOINKS FOR BIOMIMETIC BONE TISSUE ENGINEERING

Generating Fibercoll-Flex-N® collagen-hydroxyapatite bioprints with tunable mechanical properties

FIBERCOLL
FLEX-N®

TECHNICAL NOTE

THE NEED

Regenerating functional bone tissue requires biomaterials that replicate the native structure both compositionally and mechanically. Bone is a natural composite comprising mineralized collagen fibrils and inorganic hydroxyapatite (HAp), with nanoscale organization across hierarchical levels that enable flexibility and stability.[1, 2, 3] Replicating this complexity in 3D bioprinting remains challenging due to limitations in scaffold bioactivity, mechanical robustness, and printability—factors critical for experimental reproducibility and clinical translation.

THE SOLUTION

To address these limitations, we have developed a series of composite bioinks that enable the printing of bioactive 3D-scaffolds with tunable mechanical features for bone tissue engineering applications.

By combining Fibercoll-Flex-N®, a fibrillar type I collagen bioink with high mechanical strength, with microparticulate hydroxyapatite at varying concentrations we have generated a flexible, viable system that emulates the organic-inorganic interplay of native bone structure.

THE IMPACT

Fibercoll-Flex-N® + HAp composites solve critical limitations in current bone scaffold materials and have the potential to accelerate the development of next-generation regenerative therapies for complex bone defects. The system supports high-throughput fabrication of customized scaffolds for preclinical studies in osteoconduction, mineralization, or co-culture strategies with stem cells.

This broad range of applications positions Fibercoll-Flex-N® + HAp composite bioinks as a promising foundation for developing advanced in vitro bone models and translational grafts, aligning closely with the structural and functional demands of regenerative medicine and tissue engineering research.

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1. Setting up a ready-to-use protocol for Fibercoll-Flex-N® + HAp composite bioinks

Fibercoll-Flex-N® bioink consists of complex collagen type I fibers that need to be neutralized before cell loading. We have added 5 x 10^6/ml L929 cells (immortalized murine fibroblasts) to the collagen-HAp suspension to generate ready-to-use composite bioinks for 3D bioprinting.

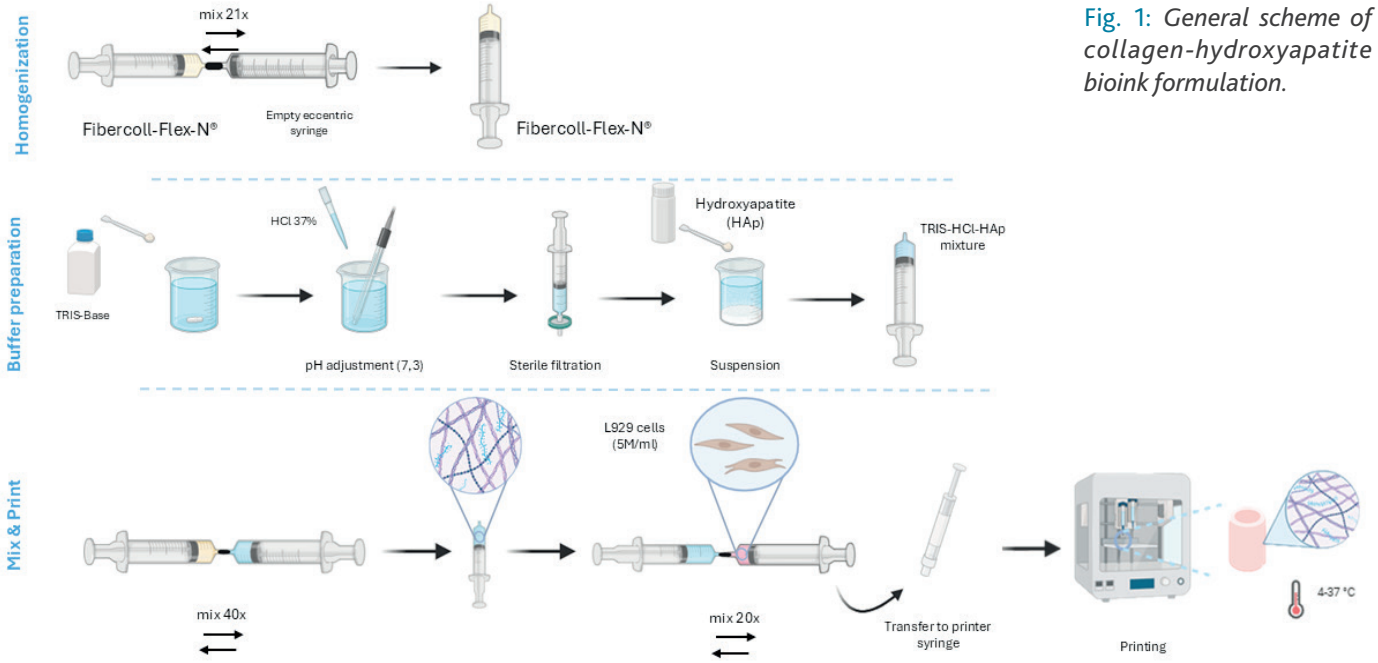

Fig. 1: *General scheme of collagen-hydroxyapatite bioink formulation.*

2. Establishing optimal bioprinting conditions

To test printability and shape fidelity of different Fibercoll-Flex-N®+ HAp composite inks we have printed* several 3D scaffolds with 4% Fibercoll-Flex-N® bioink alone as well as mixed with three different HAp concentrations (2%, 4%, and 8% w/w).

Formulation	Construct form	Pressure [kPa]	Speed [mm/s]	Pattern
Fibercoll-Flex-N® 4%	Cylinder (10 x 10 mm)	70	5	Grid
	Cube (20 x 20 x 3 mm)	90	5	Gyroid
Fibercoll-Flex-N® 4% + HAp 2%	Cylinder (10 x 10 mm)	90	3	Grid
	Cube (20 x 20 x 3 mm)	120	5	Gyroid
Fibercoll-Flex-N® 4% + HAp 4%	Cylinder (10 x 10 mm)	110	1	Grid
	Cube (20 x 20 x 3 mm)	150	3	Gyroid
Fibercoll-Flex-N® 4% + HAp 8%	Cylinder (10 x 10 mm)	135	1	Grid
	Cube (20 x 20 x 3 mm)	155	3	Gyroid

**A 22G needle was used for printing on a BioX printer (Cellink, Sweden).*

Scaffold format was chosen from the bio-printer library from the printer with a 25% infill.

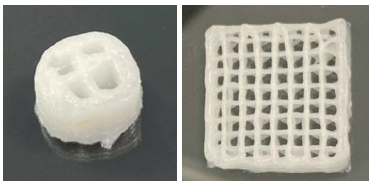

Fig. 2: *Examples of 3D scaffolds printed with Fibercoll-Flex-N® 4% + HAp 4% bioink.*

Cylinder (left) and cube (right) with high shape fidelity.

3. Analysing the tunable rheology properties of the composite bioinks

We have compared the rheology properties—elastic modulus, viscous modulus and viscosity—of three different 4% Fibercoll-Flex-N® + HAp composite bioinks (formulation see above) and the 4% Fibercoll-Flex-N® control. The data revealed significant differences in mechanical properties of the composites and the collagen control. We also observed a positive correlation between an increasing HAp concentration with a higher elastic modulus and higher viscosity.

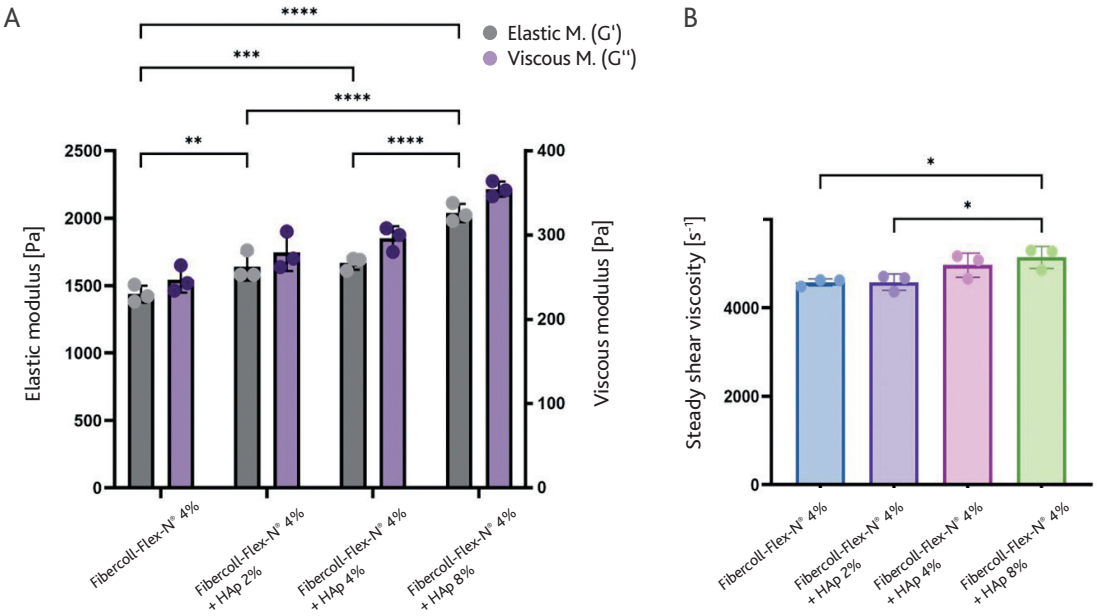

Fig. 3: *Rheology data of the four formulations.*

A) Measurement of elastic and viscous modulus with significant differences in elastic modulus between the control (Fibercoll-Flex-N® 4%) and between groups.

B) Viscosity measurements show significant differences between the highest HAp concentration bioink and the one containing 2% HAp and the control bioink.

4. Evaluating cell viability of the composite bioprints

Cell viability of the composite bioprints was evaluated in cell culture experiments with Fibercoll-Flex-N® 4% + HAp 8% in comparison to the control (Fibercoll-Flex-N® 4%). The scaffolds were printed at the same size as the ones used for rheological tests. The bioprints, containing 5 x 10^6/ml L929 cells were checked via Live/Dead Assay® at day 1 and 5. Both scaffolds showed a high amount of live cells with optimal morphology and no observable cytotoxic effects from HAp.

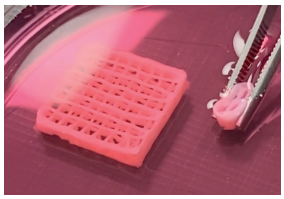

Fig. 4: *Elastic scaffolds printed with Fibercoll-Flex-N® 4% + HAp 4% bioink in culture media.*

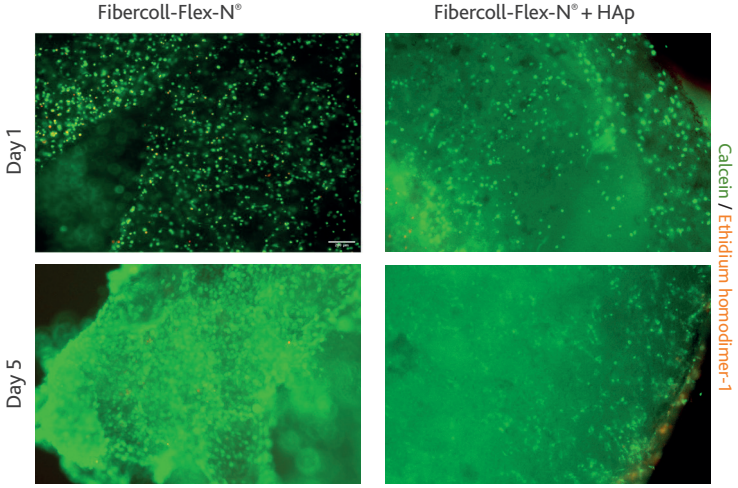

Fig. 5: *Live/Dead Assay® (Sigma Aldrich) composite images of the Fibercoll-Flex-N® scaffolds with and without HAp 8%, taken with a fluorescence microscope at day 1 and 5 of culture.*

Both scaffolds had a high live versus dead cell ratio.

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REFERENCES

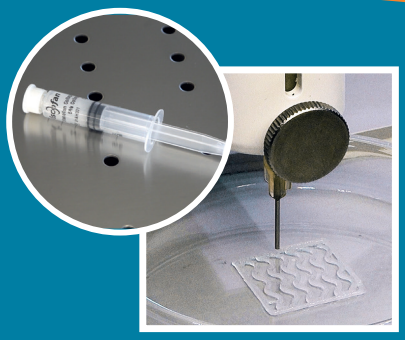
1. *Thrivikraman, G., Athirasala, A., Gordon, R., Zhang, L., Bergan, R., Keene, D. R., Jones, J. M., Xie, H., Chen, Z., Tao, J., Wingender, B., Gower, L., Ferracane, J. L., & Bertassoni, L. E. (2019). Rapid fabrication of vascularized and innervated cell-laden bone models with biomimetic intrafibrillar collagen mineralization. Nature communications, 10(1), 3520. https://doi.org/10.1038/s41467-019-11455-8*
2. *Yu, L., & Wei, M. (2021). Biomineralization of Collagen-Based Materials for Hard Tissue Repair. International journal of molecular sciences, 22(2), 944. https://doi.org/10.3390/ijms22020944*
3. *Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012;40(5):363-408.*

SUMMARY

Fibercoll-Flex-N® bioink consists of pure type I collagen fibers with high mechanical strength. By combining it with HAp in a composite bioink system, we have created a robust and flexible bioprintable platform for bone tissue engineering. We could tune the mechanical and rheological properties of the composite bioprints by using different HA concentrations, thereby modulating the strength of the scaffolds. A step closer to natural bone composition, these 3D scaffolds offer robust cell viability and high print resolution.

BENEFITS

- Tunable mechanics & shape fidelity superior to traditional collagen bioinks
- Improved osteogenic differentiation potential due to HAp content
- Compatibility with 3D bioprinting for creating patient-specific, anatomically accurate bone grafts
- Promising in vitro cell viability and proliferation, indicating strong regenerative potential



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FIBERCOLL FLEX-N®

Easy 3D model printing
with encapsuled cells at physiological conditions

- Pre-neutralization for cell bioprinting
- 2-3% final collagen concentration
- Easy API incorporation (e.g., growth factor)
- Fibrillar bovine type I collagen
- Printable from room temperature to 37°C
- No chemical crosslinking
- No gelification step required