Comparative Structural and Functional Analysis of Micronized Collagen-Based Scaffolds

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INTRODUCTION

Micronized collagen-based bioscaffolds are increasingly used in clinical applications for wound repair and soft tissue regeneration.[1] Micronized products are particularly useful in irregular wound beds, or with tunneling and undermining. Because of the increased surface area contact, the particulates are thought to improve cell infiltration and proliferation. Generally, micronized collagen-based bioscaffolds are categorized into either reconstituted collagen scaffolds, or decellularized extracellular matrix (dECM) bioscaffolds.[2] While these products have been extensively characterized as sheet-based grafts, there is paucity of information surrounding the structure-function of micronized collagen-based bioscaffolds. Here, we present the findings of the first study to perform a direct structural and functional comparison of four micronized products to inform their clinical use and guide future biological evaluation.

METHODS

Test articles included micronized coarse OFM ('mOFM', Myriad Morcells™, Aroa Biosurgery Limited, Auckland, New Zealand), micronized fine OFM ('mOFMμ', Myriad Morcells – Fine™, Aroa Biosurgery Limited, Auckland, New Zealand), micronized reconstituted bovine collagen ('pRC', CellerateRX®, Sanara MedTech Inc (Texas, USA)/Wound Care Innovations LLC (Texas, USA)/Applied Nutritionals LLC (Pennsylvania,USA)) and micronized urinary bladder matrix ('pUBM', MicroMatrix®, Integra Lifesciences, New Jersey, USA), were obtained from commercial sources.

Laser diffraction
Samples were immersed in hexane and sonicated prior to analysis with the laser diffractometer (Malvern Instruments).

$$Span = \frac{D90 - D10}{D50}$$

Microtomography
Samples were mounted on a plastic straw and analyzed with a Bruker Skyscan micro-CT with voltage=54 kV, current=200 mA, exposure time=4000 mSec. Transaxial planes were obtained with InstaRecon CPR Premium 15K and Nrecon softwares. 3D rendering was performed with CTVox.

Packing density analysis
Articles were added to a pre-weighed microcentrifuge tube ($m_{initial}$) until 1ml volume was reached and then the mass was recorded (m_{final}).

$$Packing\ density\ (mg\ per\ cm^3) = \frac{(m_{final}) - (m_{initial})}{1\ cm^3}$$

Differential scanning calorimetry
Testing was conducted as described whereby linear sections of the thermogram on either side of the thermal event peak were selected to calculate the melt onset temperature (T_m °C).[3]

Proteolytic stability
Proteolytic stability was quantified for each test article (~20 mg) in the presence of collagenase (Sigma Aldrich, USA) solution (1 mL, 50 μg/mL), as previously described.[4] Proteolytic half-life ($T_{1/2}$) was determined from interpolation of the linear quadratic regressions at 50% mass remaining.

Rheometry
Rehydrated samples that behaved as viscoelastic solids were tested with a Modular Compact Rheometer with an oscillatory strain sweep test from 0.01 to 100% strain at a constant angular frequency of 1 Hz.

Fluid absorbency
Articles were added to a pre-weighed microcentrifuge tube ($m_{initial}$), incubated with 2ml PBS, agitated, centrifuged, and the supernatant was aspirated, and the tube was wicked dry. The mass of the tube was recorded (m_{final}).

$$Fluid\ absorbency\ (\%) = \frac{(m_{final}) - (m_{initial})}{(m_{initial})}$$

RESULTS

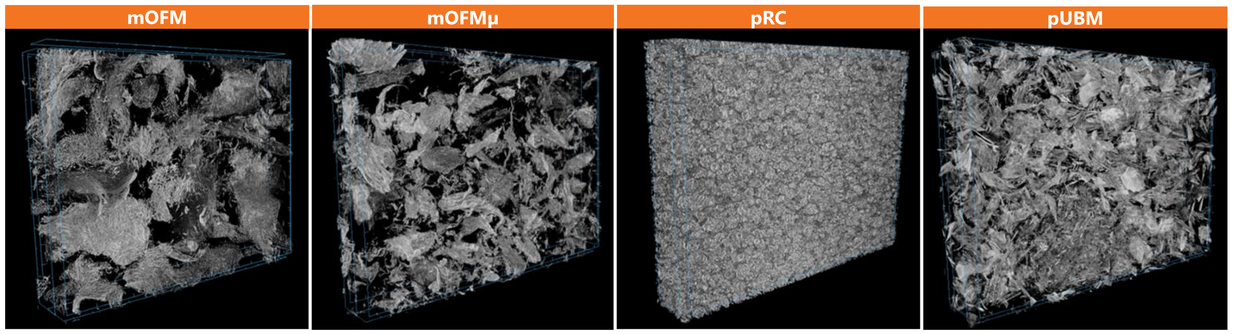

Figure 1: Representative three-dimensional micro-CT images of the test articles.

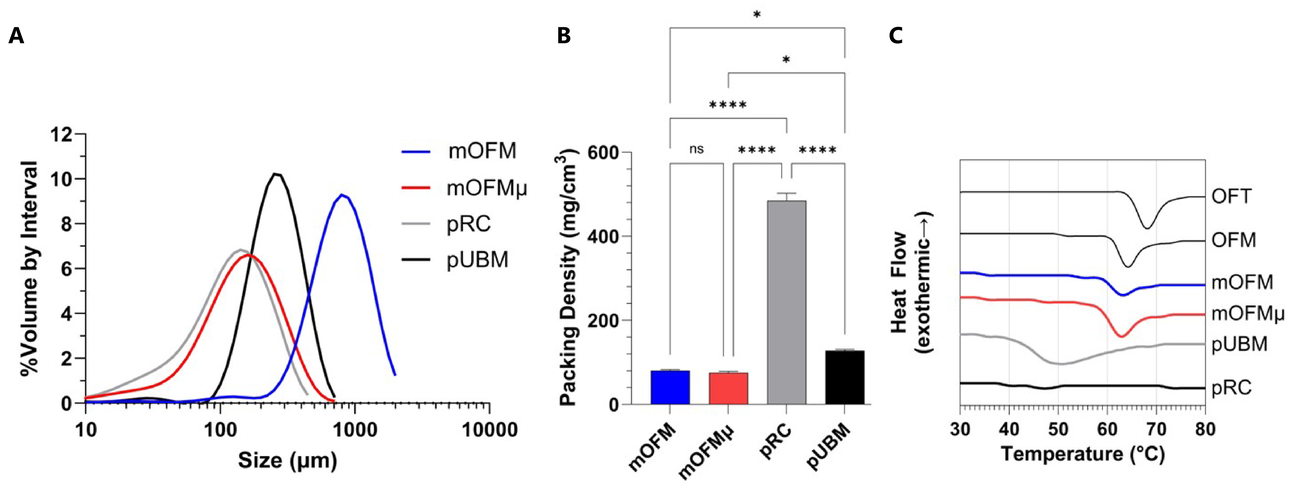

Figure 2: A. Representative particle size distribution plots. **B.** Packing density. **C.** Differential scanning calorimetry thermograms. Error bars represent the standard error of the mean. Samples ran in triplicates. Unpaired one-way ANOVA (Tukey's multiple comparison test), ns = p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

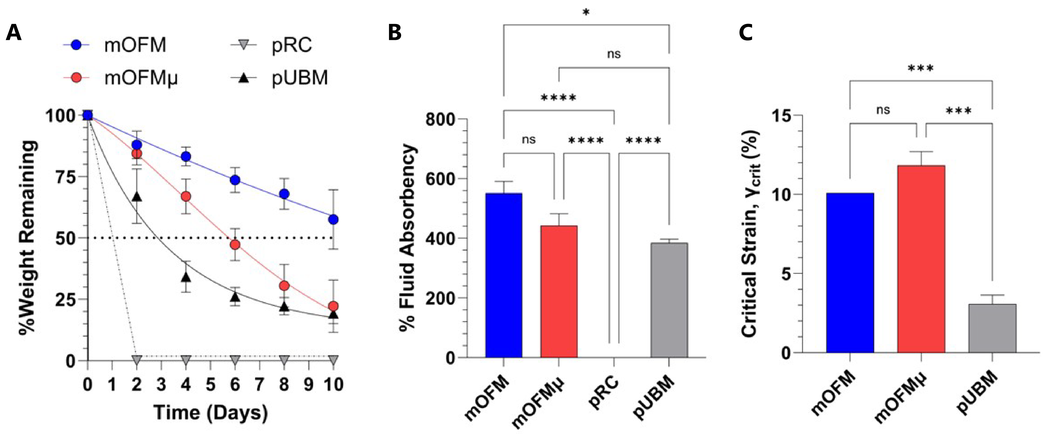

Figure 3: A. Proteolytic stability. **B.** Percentage fluid absorbency **C.** Critical strain. Error bars represent the standard error of the mean. Samples ran in triplicates. Unpaired one-way ANOVA (Tukey's multiple comparison test), ns = p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

CONCLUSIONS

- mOFM, mOFMμ have amorphous, relatively large particles with projections, the lowest packing density, highest melting temperature, highest mechanical stability, highest fluid absorbency and proteolytic resistance. Clinically, this translates to mOFM, mOFMμ requiring less product to cover a given area and fewer applications over time. Moreover, OFM particulate is stable after rehydration during handling, has a stable structure with biologically active proteins, has improved endurance in the wound environment and is able to absorb wound exudate.
- pRC has small, spherical particles, with the highest packing density, lowest melting temperature, mechanical stability, fluid absorbency and proteolytic resistance. This translates to limited wound coverage that likely requires multiple applications, an unstable structure with inert proteins, instability after rehydration during handling, rapid degradation in the wound environment and lack of wound exudate absorbance.
- These results provide meaningful indicators of scaffold persistence, integration potential, and usability, such as the number of reapplications required in a clinical context. Future studies will be needed to assess in vivo degradation kinetics, host immune responses, and regenerative efficacy in relevant preclinical models.

REFERENCES AND DISCLOSURES

1. Kallis et al., *Journal of Drugs in Dermatology*, 2018. 2. Meyer et al., *BioMedical Engineering OnLine*, 2019. 3. Karnik et al., *Biomaterials Research*, 2019. 4. Tkatchenko et al., *Dental Materials*, 2025. Research funding was provided by Aroa Biosurgery Limited (Auckland, New Zealand) and Callaghan Innovation (Wellington, New Zealand).