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Cutting-Edge Bioink Technology: Using Decellularized Skin for Enhanced 3D Bioprinting

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INTRODUCTION & AIM

Skin wounds pose a significant challenge, affecting millions worldwide. Traditional approaches to skin regeneration, ranging from autografts to tissue-engineered skin substitutes, have achieved varying levels of success. Although effective, autografts are limited by donor site morbidity and availability. On the other hand, commercial skin substitutes often suffer from poor integration and immune rejection, in addition to high costs. These challenges highlight a critical need for innovative solutions. This study aimed to develop a bioink using lyophilized rat decellularized skin (DS) for 3D bioprinting to enhance skin regeneration.

METHOD

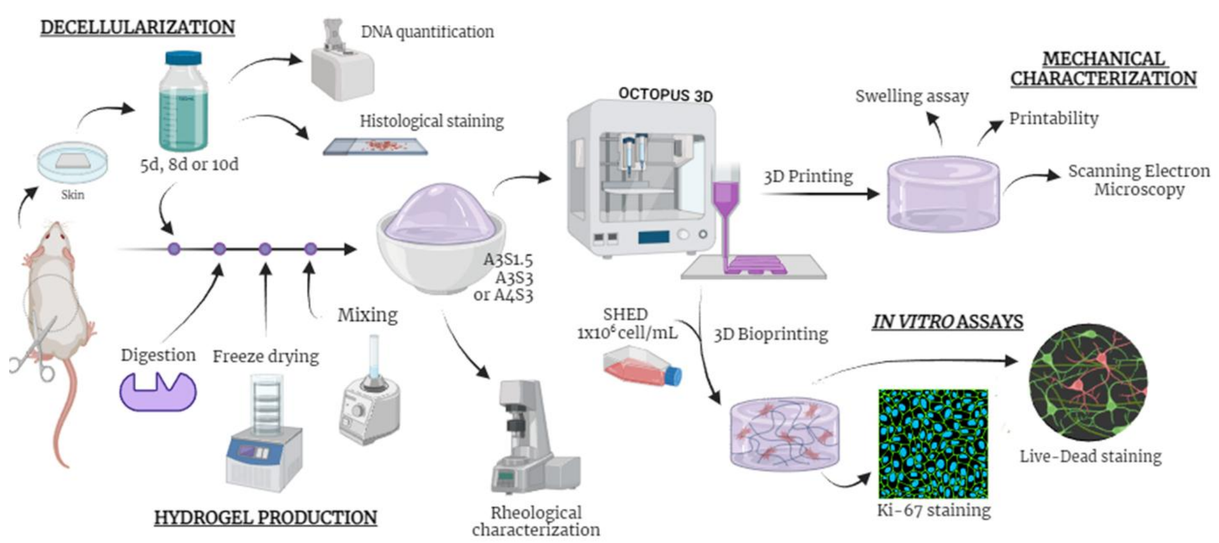

Fig. 1 – Experimental Design. Discarded rat skin decellularization process. Decellularization protocol efficiency was assessed through DNA quantification and histological staining. Skin bioink was produced by mixing 7% (w/v) porcine skin gelatin, 3 or 4% sodium alginate (A) and 1,5 or 3% lyophilized decellularized skin (S) in PBS 1X. Rheological characterization of three different concentrations (A3S1,5; A3S3; and A4S3) was realized. Hydrogels were 3D printed, SEM images were obtained and swelling assay was realized. 1x10^6 SHED/mL was added to bioink, bioprinted and analysed through Live-Dead assay and proliferation staining.

RESULTS & DISCUSSION

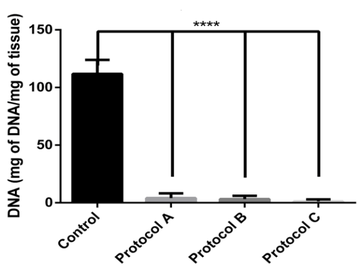

Fig. 2 - DNA quantification of three different protocols, with varying times of incubation, showed significant reduction when compared with control of native skin. ****p < 0.001.

Fig. 3 - H&E staining of samples after protocol A (A, B, C), protocol B (D, E, F), protocol C (G, H, I) and control group (J, K, L). Lines correspond to 10, 20 and 40x magnitude, respectively. **Fig. 4** - Masson's Trichrome staining. A, control group showing red staining indicating cytoplasm presence. B, decellularized group showing blue staining, indicating a higher presence of collagen than cells. **Fig. 5 -** A, protocol A; B, protocol B; C, protocol C stained with DAPI without cell nuclei. D, non-decellularized group stained with DAPI showing cell nuclei. Scale bar = 100 µm

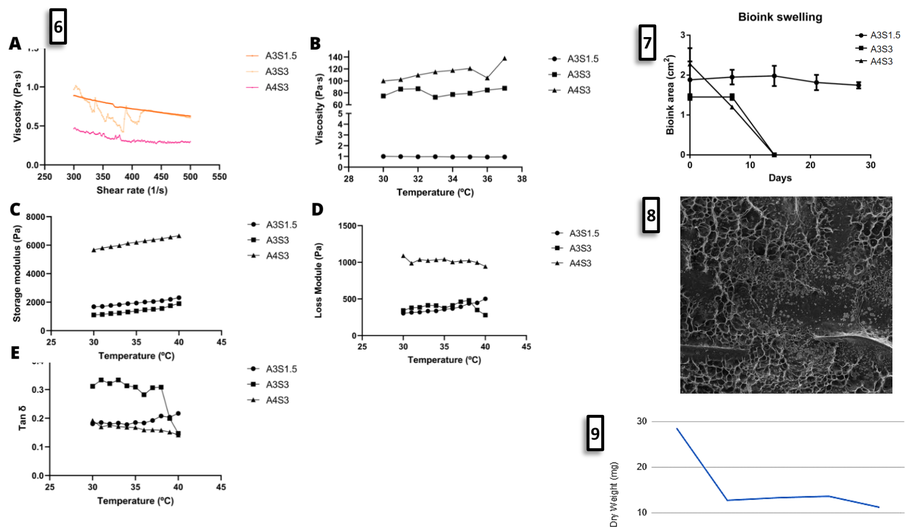

Fig. 6 - A, Viscosity vs Shear Rate. B, Viscosity vs Temperature. C, Storage modulus (G'). D, Loss modulus (G''). E, Tangent delta. **Fig. 7 -** Bioink swelling was evaluated by measuring. **Fig. 8 -** SEM image of A3S1.5 hydrogel. 3D morphology and presence of pores are enabling of 3D cell culture. Scale bar = 200 µm. **Fig. 9 -** The degradation of bioink over 28 days indicates that the bioink is biodegradable.

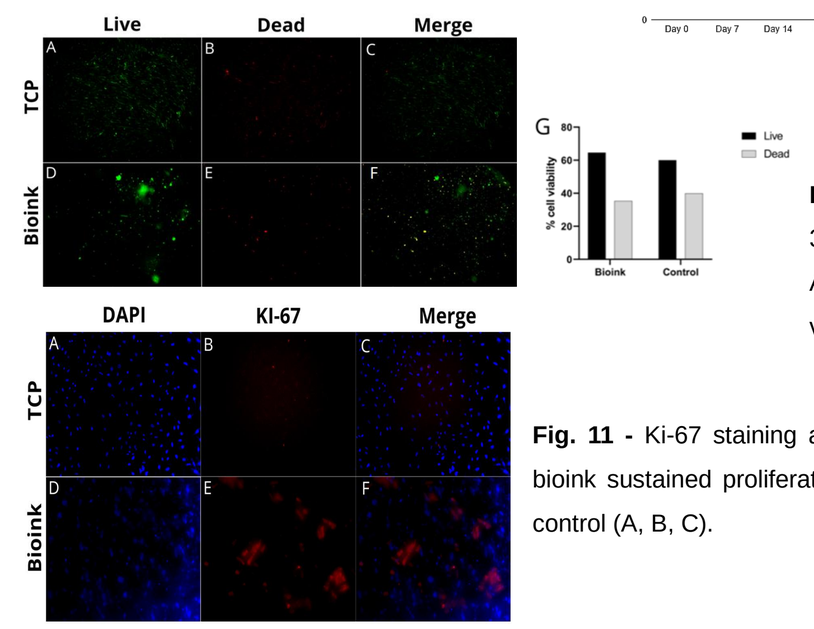

Fig. 10 - Live-Dead assay, 7 days after 3D bioprinting. A, B, C - control; D, E, F - A3S1.5 bioink. G - Quantification of viability.

Fig. 11 - Ki-67 staining after 1 day of bioprinting, indicating 3D bioink sustained proliferative status (D, E, F), as compared with control (A, B, C).

CONCLUSION

The bioink demonstrated controlled degradation over 28 days, maintaining structural integrity for a period suitable for skin regeneration. Additionally, it supported cell proliferation for 14 days, highlighting its potential as a promising solution for skin wound healing.

FUTURE WORK / REFERENCES

Future steps include optimizing bioink formulations for enhanced cell integration and testing in in vivo models to evaluate efficacy in complex wound environments.

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