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3D bioprinting stem-cells with solubilized tendon extracellular matrix (ECM) based hydrogel

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INTRODUCTION: 3D bioprinting aims to generate biological structures similar to natural counterparts such as tissues or organs in terms of their functions and morphology [1-2]. The major challenge with this technique is the choice of extra cellular matrix (ECM)-like biomaterial as a cell encapsulating agent. The objective of this research is to develop a composite hydrogel that would provide important biological cues to host cells. A new composite hydrogel system based on a mixture of natural ECM and agarose hydrogel is developed. ECM based hydrogel is derived from bovine native tendon by decellularization and enzymatic digestion procedures. Decellularized and solubilized tendon tissues contain important structural and bioactive extracellular matrix components such as collagen, which serves as anchorage sites for host cells.

METHODS: Decellularization and solubilization of bovine native tendon tissues are briefly explained in Fig. 1.

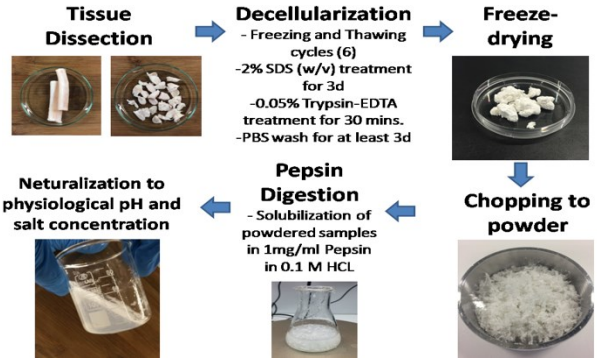

Fig. 1: Workflow to obtain decellularized and solubilized native tendon ECM.

Agarose (3%, w/v) and tendon decellularized extracellular matrix (dECM) solution were mixed with in a plate heater set to 38°C. E14 mouse embryonic stem cell suspension was introduced to agarose-dECM solution homogenously. The cell concentration in the pre-gel solution was set to 5 x 10^5 cells/ml. Extrusion of cylindrical 1 cm long cell-laden structures on pre-determined coordinates was carried out by Organovo Novogen MMX Bioprinter (Fig. 2).

RESULTS: The SEM images of tendon dECM powders indicating intact protein fibers with the absence of cells as shown in Fig. 3. High magnification images indicate collagen bundles with dense connection nodes. In addition, tile z-stack images of cell-laden structures are used to show the uniform distribution and increased viability of cells in agarose-dECM gel (Fig. 4).

Fig. 2: Bioprinting process of cell-laden hydrogel structure. (Stripe lengths are 10mm)

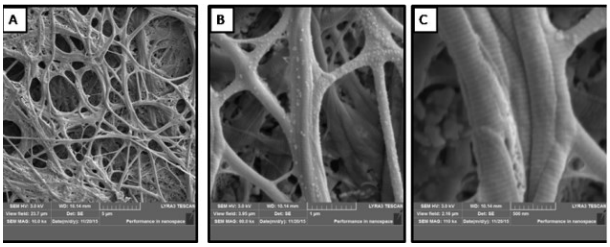

Fig. 3: SEM images of tendon dECM powders-magnifications A)10kX, B)60kX and C)110kX

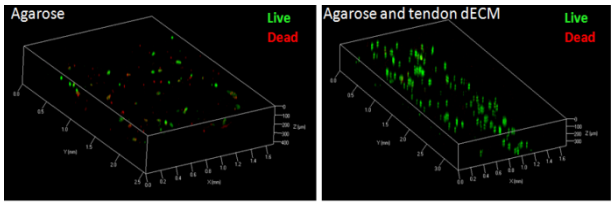

Fig. 4: Confocal images of cell-laden hydrogel constructs.

DISCUSSION & CONCLUSIONS: The results showed the biocompatibility of produced tendon dECM solution and the increased viability of MESC's encapsulated in agarose-dECM gel even after three days of incubation. Tendon dECM solution supported agarose hydrogel has a potential to be used as a cell encapsulating agent for 3D bioprinting applications.

REFERENCES: [1] S. Murphy, A. Atala (2014) *3D Bioprinting of Tissues and Organs*, Nature Biotechnology, **32**:773-785. [2] Kucukgul C. et al. (2015) *Biotech and Bioeng*, DOI:10.1002/bit.2549