Bone organoid generation based on double scaffolding

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Decellularized bone matrix (DBM) is a classic approach in bone tissue engineering based on the removal of bone tissue cells and the calcified phase using chemical, physical o enzymatic agents. The resulting matrix preserves its three-dimensional structure and biomechanical properties, providing a native microenvironment suitable for osteogenic development. However, due to the complexity of the bone structure, with this method has not been possible to obtain an experimentally reproducible bone organoid that sufficiently replicates the bone biology. Another approach to generate organoids is the use of hydrogels functionalized with adhesion peptides and morphogenetic proteins that favor the differentiation and osteogenic capacity of the organoid. In this work we developed a new bone model based on the combination of hydrogels and DBMs. We tested PVA and dextran hydrogels in combination with BMP responsive reporter cell line (BRITER) and the long-term effect of the RGD peptide addition to hydrogels evaluating osteogenic differentiation by alkaline phosphatase method and luciferase assays. We analyze by histology decellularized DBMs combined with dextran hydrogels containing BRITER cells cultured under osteogenic conditions for 3 weeks. Our results showed that this double scaffolding is able to support osteogenic differentiation as well as osseointegration, proving its potential for bone organoid generation.

Hydrogel organoid

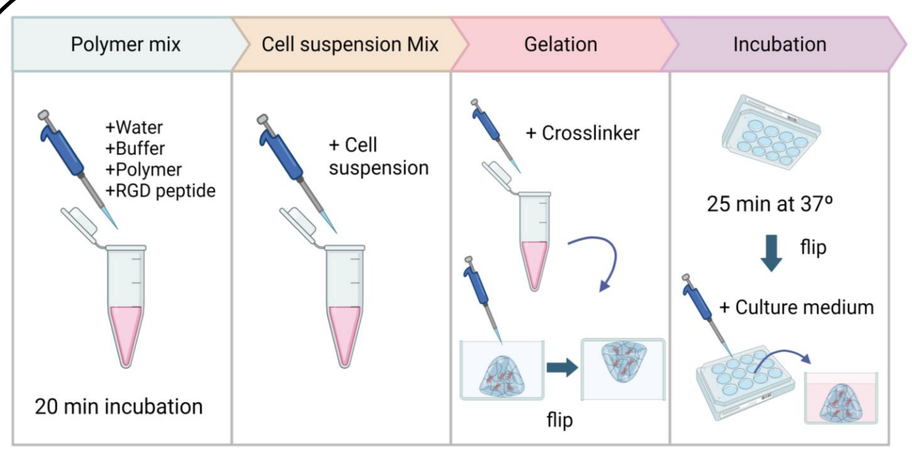

Figure 1. TrueGel3D™ Kit hydrogel protocol flowchart. Image created with BioRender.

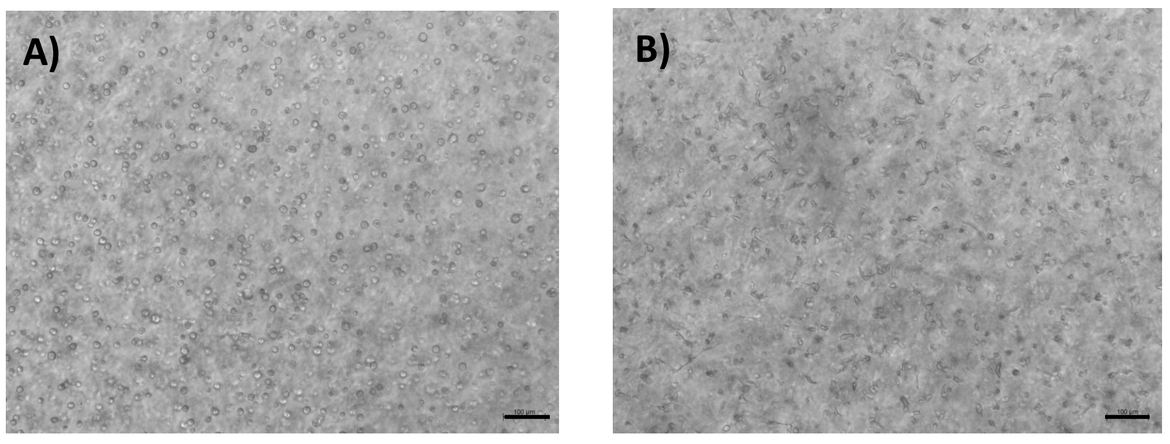

Figure 2. Osteogenic induction reporter cells of 5 days culture in dextran hydrogels. A) Hydrogel without RGD peptide in which cells show a round morphology. **B)** Hydrogel with RGD peptide, in which cells present an adherent morphology. Scale bar 100 µm.

Properties	PVA	Dextran
Gelation time	1 min	3 min
3D cell distribution	-	+

Table 1. Comparison of physical properties of PVA and dextran hydrogels. The dextran hydrogels presented a longer gelation time, which makes them more suitable for their introduction into DBMs.

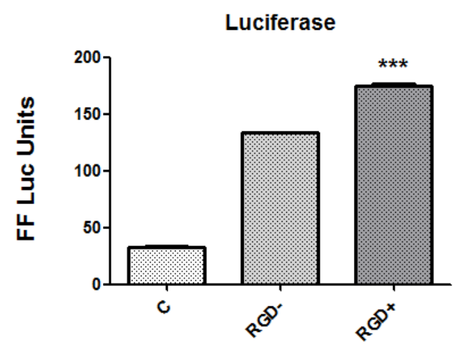

Figure 3. BMP2 dependent Luciferase activity of osteogenic induction reporter cells of 48h culture in dextran hydrogel: (C) without BMP2 stimulation, (RGD-) hydrogel without RGD peptide and (RGD+) with RGD peptide, both with 3h BMP2 stimulation.

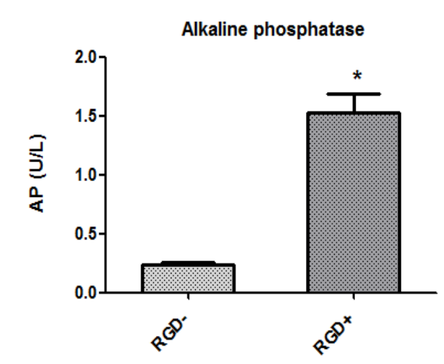

Figure 4. Alkaline phosphatase activity of osteogenic induction reporter cells of 3 weeks culture in dextran hydrogel. (RGD-) hydrogel without RGD peptide. (RGD+) hydrogel with RGD peptide.

Hydrogel + DBM organoid

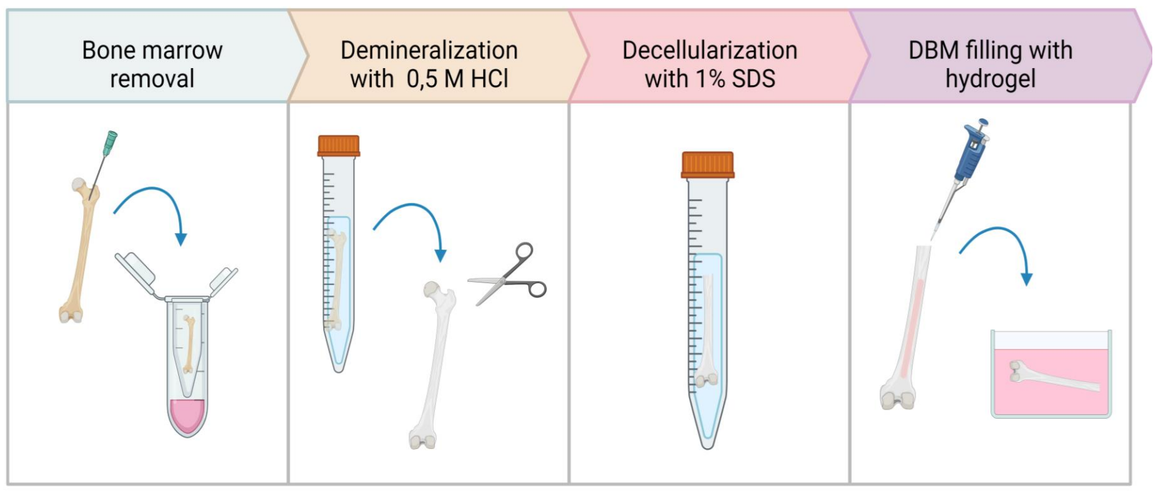

Figure 5. DBM and hydrogel organoid protocol flowchart. Bone marrow form mouse femur are removed by doing a hole in the proximal epiphysis and centrifuging. Then, the femur is decalcified with 0,5M HCl for 24 hours, the proximal epiphysis is removed, and it is decellularized with 1% SDS for 24 hours. Finally, the DBM obtained is filled with the hydrogel and transferred to a well of a plate with culture medium. Image created with BioRender.

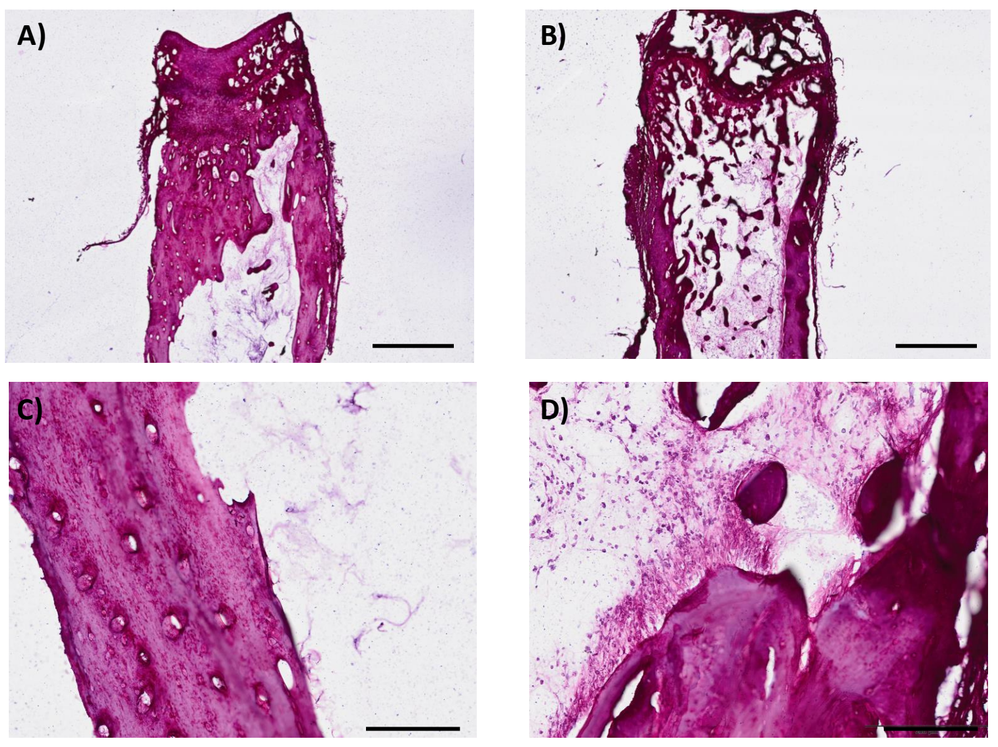

Figure 6. Histology of DBM and hydrogel combination cultured for 3 weeks under osteogenic conditions. (A, C) DBM filled with hydrogel. (B, D) DBM filled with hydrogel and osteogenic induction reporter cells. (A, B) Scale bar 500 µm and (C, D) 100 µm. Picrosirius stain.

CONCLUSIONS

Combination of DBM and RGD functionalized dextran hydrogel provides three dimensionality and promotes bone differentiation, which constitutes the bases for developing a bone organoid. In future work, this double scaffolding will be functionalized with morphogenetic proteins and different osteogenic cell lineages to create a native bone environment.

Acknowledgment

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