OSTEOGENIC POTENTIAL OF SUPRAMOLECULAR UPY-ALENDRONATE HYDROGELS: AN IN VITRO STUDY

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Introduction

Alendronate (ALN) is a bisphosphonate clinically used to treat skeletal disorders, including osteoporosis. Alendronate is incorporated in bone by the formation of noncovalent bonds with calcium present at the surface of hydroxyapatite [1]. Once osteoclasts resorb bone and take up alendronate it inhibits osteoclast activity and thereby reduces bone resorption. The binding affinity for calcium ions can also be utilized in the formation of hydrogels [2,3]. Through incorporation of alendronate endgroups gelation is achieved upon contact with calcium. Hydrogels with tunable configurations and physico-chemical properties can be used for cell or drug delivery to induce local bone repair. Promising candidates in regenerative medicine are supramolecular hydrogels based on ureido-pyrimidinone (UPy) moieties because they form dynamic non-covalent interactions [4]. This research aims to examine the ability of alendronate functionalized UPy hydrogels to support osteogenesis and osteoclastogenesis.

Methods

UPy solution containing 10 wt% UPy-ALN was mixed with cell suspension in calcium free PBS containing MSCs or PBMCs at concentrations of 10 x 10^6/ml and 100 x 10^6/ml respectively. 50 µl gel-cell suspension was extruded into calcium rich culture medium to achieve immediate gelation. Hydrogels with cells encapsulated were cultured for 4 weeks in medium supplemented with dexamethasone, ascorbic acid and ß-glycerophosphate. On day 28, whole gel mounts (n=3) were stained with CNA, OsteoSense^{TM} and Hoechst to analyze matrix formation (gel with MSCs) or stained with phalloidin and Dapi to visualize cell morphology (gel with PBMCs). To evaluate osteogenic differentiation gels (n=3) were cryo-embedded. Frozen sections were fixed, and immunohistochemistry was performed for markers RUNX-2 and Osteopontin (gel with MSCs).

Results

At the end of culture, differentiation of MSCs towards osteoblasts was achieved in UPy-ALN gels (Fig. 1A). Furthermore, matrix formation was observed with large quantities of collagen and hydroxyapatite (Fig. 1B). Regarding differentiation of PBMCs towards osteoclasts, cellular fusion was seen and multinucleated cells with an actin ring were detected (Fig. 1C). Remarkably, within certain multinucleated cells, nuclei were partly fragmented, indicating cellular apoptosis.

Discussion

UPy-ALN hydrogel demonstrated to support osteogenic differentiation and matrix formation. Interestingly, signs of apoptosis in osteoclast-like multinucleated cells were found. This might be a result of phagocytic osteoclasts internalizing UPy-ALN moieties causing ALN to inhibit the mevalonate pathway, which is necessary to function properly [5]. Thereby, UPy-ALN gel seems to allow bone formation while suppressing resorption. In combination with the ease of cell encapsulation and gelation upon injection in a calcium rich environment this gel has potential to be applied locally. This could be useful in for example early-stage OA when lesions occur in the subchondral bone. Although UPy-ALN gel has insufficient stiffness as substitute for large bone defects it might be used as osteoinductive material together with other materials, such as calcium phosphate. Different hydrogel formulations could be investigated to change its stiffness. As a next step, UPy-ALN gels will be injected in a bony environment to further explore its potential as biomaterial for bone regeneration strategies.

References

1. Drake et al, Mayo Clin Proc, 83: 1032–1045, 2008
2. Lopez-Perez et al, Macromolecules, 50: 8698–8706, 2017
3. Van Erk et al, Biomaterialia, 20: 75–87, 2022
4. Goor et al, Chem Soc Rev, 46: 6621-6637, 2017
5. Fisher et al, PNAS, 96: 133-138, 1999

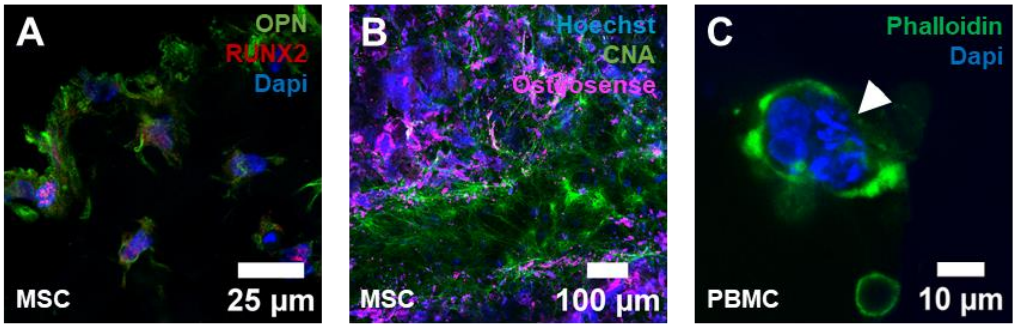

Figure 1 – (A) UPy-ALN gel section with MSCs encapsulated, showing cells positive for osteoblast markers OPN and RUNX-2. Whole gel mount (B) visualizing collagen (CNA), hydroxyapatite (OsteoSense^{TM}) and nuclei (Dapi) in gel with MSCs and (C) showing an osteoclast-like multinucleated cell with an actin ring and nuclear fragmentation (white arrow) in gel seeded with PBMCs.