

Lab Report

“Bone Tissue Engineering”

M.Sc. BMT, Implantology
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I hereby declare that my elaboration was made independently and is not a copy of an existing protocol.

Date: June, 8th 2022

Signature:

Introduction

In the field of regenerative medicine, bone tissue engineering (BTE) provides a promising option for the treatment of several pathologies related to lacking bone that can be caused either due to diseases, injuries, or genetic disorders [1]. Currently, autografting is taken as gold standard for treatment [1]. However, this option shows several limitations, such as limited graft supply, high donor site morbidity, and the cause of secondary damages on a patient [1]. With a combination of stem cells, scaffolds and growth factors, BTE is able to regenerate bone, and additionally to overcome the limitations of autologous bone grafting [1]. To enable optimal conditions for bone regeneration, the selection of the right scaffold material is crucial [2]. A parameter often used for evaluation of potentially suitable scaffold materials is the enzyme alkaline phosphatase (ALP) that functions as marker for osteoblastic cell activity, and therefore indicates bone development [3]. ALP plays an important role in the regulation of bone mineralization by degrading the mineralization inhibitor pyrophosphate enzymatically [3]. Stem cells can either be cultured on a monolayer (2D) or in a scaffold structure (3D). While 2D BTE provides easy and good results, 3D BTE is necessary to mimic physiological conditions, thus to enable a more complex environment in vitro that should be similar to in vivo conditions [4].

In this practical, ALP activity was investigated for human jaw periosteal cells (JPCs) seeded on beta tricalcium phosphate (β -TCP) scaffolds and on a monolayer in a 6-well plate. For both, the 3D and 2D cell approach, JPCs were cultured in control (CO) and osteogenic (OB) medium to analyze different influences on the osteogenesis of JPCs.

Methods

Bone-specific ALP activity of the 2D and 3D samples was quantified by using an ALP assay kit (*SensoLyte® pNPP Alkaline Phosphatase Assay Kit "Colorimetric", Anaspec*) that is based on colorimetric detection. *p*-Nitrophenyl phosphate (pNPP) contained in the test kit turns yellow due to dephosphorylation induced by ALP present in the respective samples. The absorbance of the sample is measured by a microplate reader at a wavelength of 405 nm.

For normalization of the amount of cells in the ALP assay, additionally the total protein amount of ALP in the 2D and 3D samples was detected by using the *Protein Assay Kit* from *Thermo Scientific*. This assay is based on the reagent bicinchoninic acid (BCA)

that enables colorimetric detection of Cu^+ in a highly sensitive manner. In the presence of alkaline medium, Cu^{2+} is reduced to Cu^+ by proteins, here the ALP. At a wavelength of 550 nm the binding of one Cu^+ on two BCA molecules, giving the sample a purple color, can be detected and quantified.

The detailed steps that the lab procedure was followed are written down in the given handout "Bone Tissue Engineering". The albumin standard was prepared by the supervisors because the albumin standard prepared by the practical group was not working.

Results

In Table 1 the measurement values, their means and standard deviations (SD) of the BCA and ALP assays are listed. Moreover, it shows the calculated ratio of the ALP to the amount of total protein in percent. Since the ALP concentration in the ALP assay was detected in a solution diluted by a factor 1:5, but the measured total protein concentration was detected undiluted, the obtained measurement values for the ALP assay were multiplied by a factor of 5.

Figure 1 depicts the mean values of ALP measurement normalized to the total protein amount measured in the BCA assay with their standard deviations in percent. The 2D samples are represented by the blue bars, and the 3D samples by the orange ones. With two-tailed t-tests, it was checked whether the mean values of the CO and OB samples between the 2D and 3D samples, and the CO and OB samples themselves within the 2D and 3D samples differed significantly. Before every t-test, a F-test was done to decide whether the variances between the means were equal or unequal.

It could be observed that the ALP activity in the 2D medium was significantly higher than in the 3D scaffolds. Moreover, it was detected that the ALP activity for cells cultured in the osteogenic 2D sample was not significantly higher than the activity detected for cells that were cultured in the 2D control medium. For the 3D samples the ALP activity was significantly higher in the sample where the JPCs were cultured in osteogenic medium than in the control medium.

Table 1: Measurement values, means and standard deviations (SD) of total protein concentration, ALP concentration and their calculated ratio

Sample		Single Values			Mean	SD
Total Protein [mg/ml]	2D CO	0.434	0.461	0.472	0.456	0.020
		0.423	0.625	0.374	0.474	0.133
	3D CO	0.381	0.389	0.386	0.385	0.004
		0.373	0.410	0.403	0.400	0.020
ALP [ng/ml]	2D CO	941.780	937.210	975.860	951.617	21.119
		1390.650	1605.525	1118.835	1371.670	243.900
	3D CO	93.080	118.850	156.255	122.728	31.766
		21.595	29.490	49.855	33.647	14.581
Ratio ALP/ Total Protein [ng/ml] [%]	2D CO	0.217	0.203	0.207	0.209	0.007
		0.329	0.257	0.299	0.295	0.036
	3D CO	0.024	0.031	0.040	0.032	0.008
		0.006	0.007	0.012	0.008	0.003

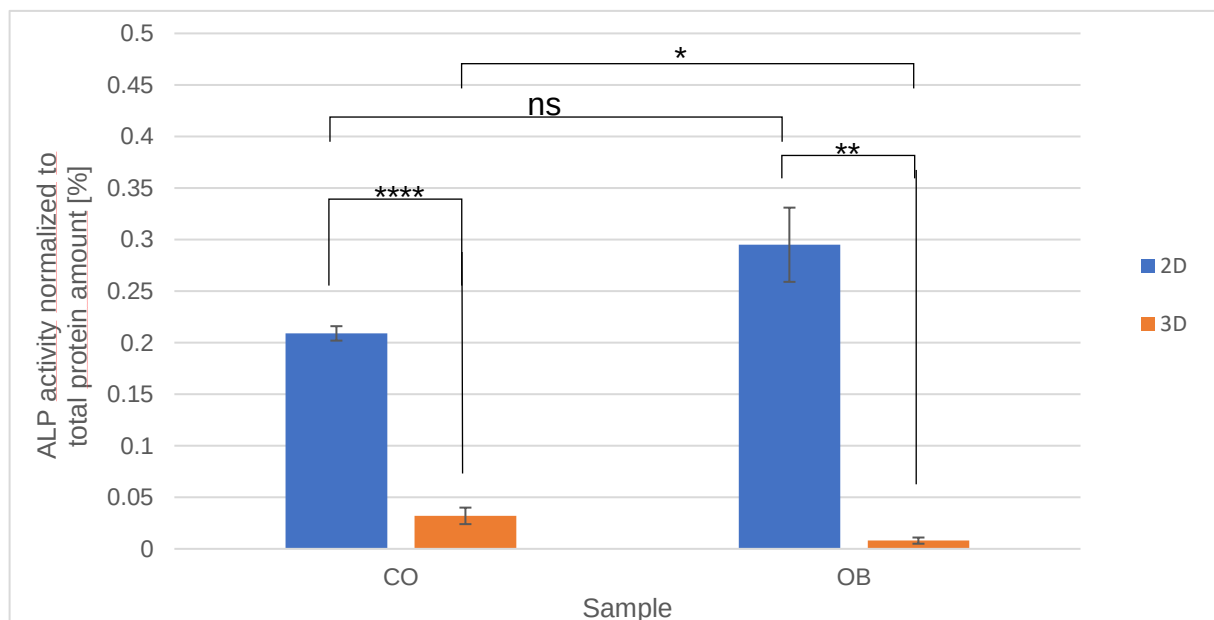


Figure 1: ALP activity normalized to total protein amount for 2D (blue) and 3D (orange) samples with CO and OB medium each. Significance was tested with t-tests, ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$

Discussion

In this practical ALP activity was investigated for JPCs cultured in 2D medium and 3D scaffolds, in CO and OB medium each, to evaluate the suitability of different environments regarding bone formation. The practical results showed that ALP activity was higher in the 2D than in the 3D samples. Generally, it would be favorable if the ALP activity was higher in the 3D cultures because these scaffolds should provide a suitable environment for bone growth. Potential reasons that this was not the case in this practical are either mistakes during preparation of the samples, such as wrong pipetting, or that the β -TCP scaffold is an inappropriate environment for culturing JPCs. Furthermore, the three 3D samples for CO and OB each, were prepared in the same tube and just pipetted in three different wells for the measurement. Therefore, it did not really make sense to recalculate the mean values of the three wells each. Usually, osteogenic medium should enhance bone formation, and thus increasing the ALP activity. However, only in the 3D samples, the ALP activity in the OB medium was significantly higher than in the CO medium. For the 2D samples there was no significant difference in the ALP activity between the CO and OB samples. This might also be due to mistakes during sample preparation. Further factors that could have influenced the results are that the BCA assay was not measured after 30 min as recommended, but after approximately 45 min, what might have led to higher measurement values due to the ongoing reaction. Moreover, the ALP standards were not prepared by ourselves. In conclusion, this practical showed that different environments influence the osteogenesis of JPCs. Therefore, it is crucial to obtain further detailed information about different scaffold materials and the media the cells are cultured in.

Literature

1. Qi, J., et al., *Current Biomaterial-Based Bone Tissue Engineering and Translational Medicine*. Int J Mol Sci, 2021. **22**(19).
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