# Human Osteoblast Culture on TGF-ß Coated Polycaprolactone Scaffolds in Dexamethasone Conditioned Media

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**Statement of Purpose:** Vascular calcification is a significant phenomenon associated in late stage atherosclerosis, which is the leading cause of mortality and morbidity in North America. Current animal models of atherosclerosis lack calcium and there is an urgent need to model atherosclerotic calcification for the purpose of both understanding and treating this disease. The overall goal of this study is to use a tissue engineering scaffold to generate atherosclerotic calcification in an animal model. This study focused on establishing calcium deposits on 3D structural poly(ε-caprolactone) (PCL) scaffolds. The strategy is to culture Human Normal Osteoblasts (HNOsts) on TGF-ß coated PCL scaffolds in dexamethsone (Dex) supplemented media.

**Methods:** Human Normal Osteoblasts (HNOsts) were used at Passage 3, and were cultured on poly(ε-caprolactone) (PCL) (IV=1.14dL/g; Mw=128K Dalton) scaffolds. PCL scaffolds were fabricated using the vibrating particle technique. Cell culture media used was α-MEM media supplemented with 7% FBS, 100µg/ml L-ascorbic acid, and 5mM ß-glycerophosphate (osteogenic media or OM) with or without the addition of Dex. Ten test groups were used: 0ng TGFß coating on PCL in OM (no Dex, 0ng); 5ng TGFß coating on PCL in OM (no Dex, 5ng); 10ng TGFß coating on PCL in OM (no Dex, 10ng); 50ng TGFß coating on PCL in OM (no Dex, 50ng); 100ng TGFß coating on PCL in OM (no Dex, 100ng); 0ng TGFß coating on PCL in Dex conditioned media (Dex, 0ng); 5ng TGFß coating on PCL in Dex conditioned media (Dex, 5ng); 10ng TGFß coating on PCL in Dex conditioned media (Dex, 10ng); 50ng TGFß coating on PCL in Dex conditioned media (Dex, 50ng); and 100ng TGFß coating on PCL in Dex conditioned media (Dex, 100ng). Images of HNOst cell cytoplasma (using Eosin) were captured after cells were cultured on scaffolds for 30 days. ALP activity and calcium content in cellular scaffolds were measured in the ten study groups at Day 21. **Results:**

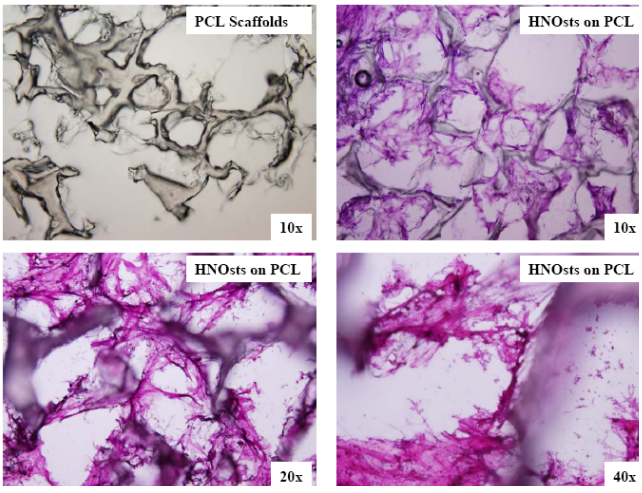


Fig. 1. HNOst cell spreading in PCL scaffolds at magnifications of 10x, 20x, and 40x. Eosin stains cell cytoplasma as pink.

PCL scaffold structure and HNOsts cell distribution are shown in Fig. 1 at different magnifications. HNOsts were seen spreading extensively inside the scaffold.

ALP activity of HNOsts on "no Dex 5ng" group surpassed "no Dex 10ng" at Day 21 ($p<0.05$) (Fig. 2). "Dex 0ng" exceeded "no Dex 0ng" ($p<0.05$). "Dex 5ng" had higher ALP activity than "Dex 10ng" and "Dex 50ng" at Day 21.

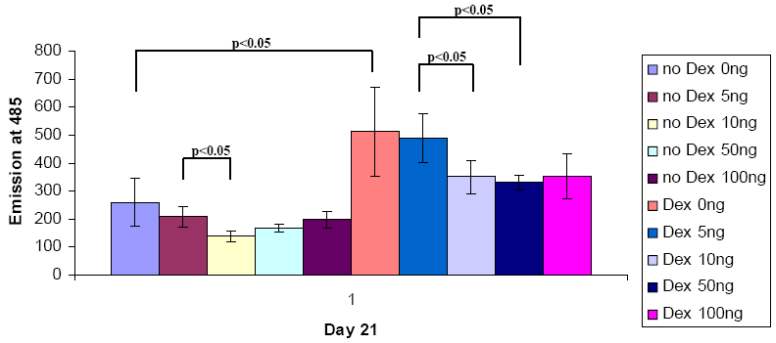


Fig. 2. ALP activity of HNOsts on TGFß coated PCL scaffolds at Day 21.

After 21 days of culture, HNOsts on "Dex 0ng" group had more calcium deposits than "no Dex 0ng" group ($p<0.05$) (Fig. 3). "Dex 5ng" established more calcium than "Dex 50ng" and "Dex 100ng" ($p<0.05$).

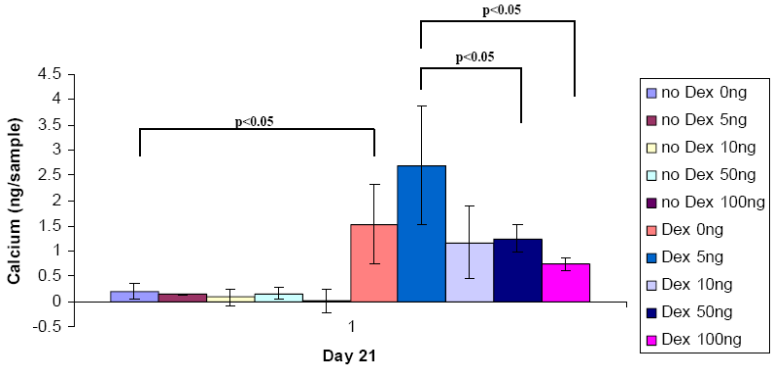


Fig. 3. Calcium Measurement of HNOsts on TGFß coated PCL scaffolds at Day 21.

**Conclusions:** PCL scaffolds support HNOsts ingrowth. Dex has a more significant effect on calcium establishment in HNOst culture than TGF-ß. Lower dosage of TGF-ß (5ng coating) works better than higher dosages (50ng and 100ng coating) in terms of calcification.

**References:** (1) Chim H *et al.* J Biomed Mater Res A, 2003, 65(3): 327-335. (2) Lin J *et al.* J Tissue Eng Regen Med, 2007, 1(3): 211-217.