In Vitro Bone Growth Responds to Tissue-Level Mechanical Strain in Three-Dimensional Polymer Scaffolds

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Introduction: Mechanical stimulation plays a key role in healing and remodeling of bone tissue and is used in regeneration of bone tissue in vitro. Macroscopic compression of 3-D seeded polymeric scaffolds in vivo increases mineralization [1]. Earlier, we found that compression of 3-D polymer scaffolds increases protein levels of several bone markers and calcification [2]. However, calcification is uneven through the scaffold. We hypothesized that differences in local strains within the scaffold predict the differences in the local biological response. In this study, we calculated the local strain distribution due to compression within polymer scaffolds, seeded them with cells and determined whether local strains would predict bone nodule formation as detected from micro-CT scans after culturing under conditions of cyclic compression.

Materials and Methods: Twelve porous cylindrical scaffolds (9 mm diam, 4 mm high, 90% porous) were made from poly-L-lactic acid (PURASORB, Purac Biochem, Gorinchem, Netherlands) [2]. Each scaffold was scanned with a μCT scanner using a resolution of 15 μm (μCT40, Scanco Medical AG, Zürich, CH). The voxel data was used to calculate the local strains inside each scaffold due to compression, using micro-Finite Element (μFE) analysis (Scanco Medical AG, Zürich, CH).

Osteoblastic cells were grown from rat tibia bone chips and cultured in DMEM with 10% FCS, 1% L-glutamine, 1% ascorbic acid, 1% dexamethasone, 1% β-glycerophosphate and 1% antibiotic and antifungal solution. Six scaffolds (B1) were seeded with 2.5x10⁶ cells and six (B2) with 5x10⁶ cells at passage 2. Seeded scaffolds were kept for two (B1) or three (B2) weeks in static conditions. After this period, the scaffolds were cyclically compressed (1 Hz, 60μm ~1.5% strain) one hour per day, for a period of one week [2].

After culturing, each scaffold was re-scanned with the μCT scanner to determine the distribution of mineralized nodules. The μFE data was used to determine the largest principal strain at the pore surface, averaged over a small volume (30 μm radius), at the site of each mineralized nodule. To compare between strains at mineralized and non-mineralized sites within single scaffolds, we also determined the largest principal strain at the pore surface, averaged over a small volume, at 200 regularly spaced non-mineralized sites.

Results: Compressing gave a highly non-homogeneous distribution of local strains at pore surfaces, with areas of compression and tension and of high and low strain magnitudes in close proximity (Fig. 1).

After culture, the average number of mineralized nodules was 36±18SD (B1) and 59±24SD (B2). The average value of the mean pore strains at sites with a mineralized nodule was two to four times larger than that at sites without a nodule for each individual scaffold (Fig. 2). The difference in strains was highly significant (p<0.001, Wilcoxon signed rank test).

Discussion: This study shows that inside cyclically compressed 3D scaffolds, local mechanical strains at pore surfaces differ between sites at which bone cells proceed to form mineralized nodules and sites at which bone cells do not proceed to form these nodules.

To our knowledge, this is the first study directly linking local mechanical strains to local new bone formation in a 3D environment. Before the experiment, we calculated the mechanical strains at the pore surfaces inside each scaffold. When comparing the strain magnitude between sites in the scaffolds that did and did not form mineralized nodules, we found two to four times larger average strains at sites that formed a nodule. This suggests that bone cells in a 3-D environment are sensitive to the absolute magnitude of the local surface strain. Direct comparison between in vitro bone formation in 3-D scaffolds and in vivo bone formation is difficult. However, the range of average surface strains at sites in our study where a nodule formed (<0.1%) is comparable to strain levels that induce formation of mineralized nodules on deformable 2-D membranes with similar cells and culture conditions [3].

We used mineralized nodule formation as measured by μCT to quantify mineralized matrix deposition. Bone nodule assays are a common marker of osteoblast differentiation. In addition, restricting our analysis to discrete mineralized nodules excluded dispersed calcification, e.g. dystrophic calcification.

The method in this study allows researchers to investigate the effect of macroscopic compression on a local level. This is an improvement on the current situation, where investigations concentrate on the average effect on bone formation from macroscopic strains. By improving our understanding of the relation between local strains and local bone formation, this method can help in the rational design of scaffold micro-architecture. Such scaffolds might guide bone growth in vitro by delivering better strain regimes.


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