

## The Effects of Decellularisation on the Mechanical Properties of Bone, and Subsequent Recellularisation of the Samples

Categories:

Biomechanics and Bone Quality

Mechanobiology

*Poster Sessions, Presentation Number: MO0148*

*Session: Poster Session III*

*Monday, October 12, 2015 12:30 PM - 2:30 PM, Washington State Convention Center, Discovery Hall - Hall 4BC*

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Regenerative medicine strategies involving decellularised extracellular matrix scaffolds are developing fast and, in particular, decellularized bone has been proposed for bone tissue engineering. This study aimed to establish decellularisation and recellularisation protocols and to measure the Young's modulus and pore size of the decellularised trabecular bone samples. Twelve bovine cancellous proximal femur samples (7mm x 7mm x 2mm) were decellularised by six cycles of overnight incubation at 37°C using two protocols: A – 10mM Tris, 1mM EDTA, 0.1% v/v Triton X-100 and B – method A plus 0.5% w/v trypsin. Decellularisation was confirmed by the absence of DNA staining with DAPI both by detecting any DNA remaining on the bone matrix spectrofluorometrically, and by microscopic examination. Young's modulus was determined before and after incubation through compression testing at 1 mm/s up to 400N (8.16MPa). The porosity of the bone samples before and after decellularisation was measured using a mercury porosimeter. Recellularisation using HOS cells (seeded at  $5 \times 10^5$  cells per  $\text{cm}^2$  bone) progressed for up to 3 weeks in DMEM supplemented with L-ascorbic acid,  $\beta$ -glycerophosphate, dexamethasone, FCS, PEST, and NEAA. Bone samples were placed onto non-adherent dishes and adherent dishes. The extent of recellularisation was compared in static and dynamic culture conditions using a roller incubator set at 15 rpm to effect dynamic conditions. DAPI staining revealed that protocol B removed all measurable DNA from the bone samples (Figure 1). Decellularisation did not affect Young's modulus (Figure 2). Pore diameters did not differ with decellularisation and were in the ideal range for cell growth. Mean ALP activity (Figure 3A) and MTT reduction (Figure 3C) was greater on the adherent surface than on non-adherent surface albeit non-significantly. There was no significant difference between static and dynamic conditions in ALP activities between 3 and 7 days (Figure 3B). Data suggests that cells proliferated more readily when samples were placed in adherent dishes (Figure 3D). This work has established appropriate protocols to make donor bone scaffolds with appropriate porosity to allow reseeding with human bone cells. These could be used to repair bone defects in recipient patients.

**Disclosures:** None

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## ATTACHMENTS

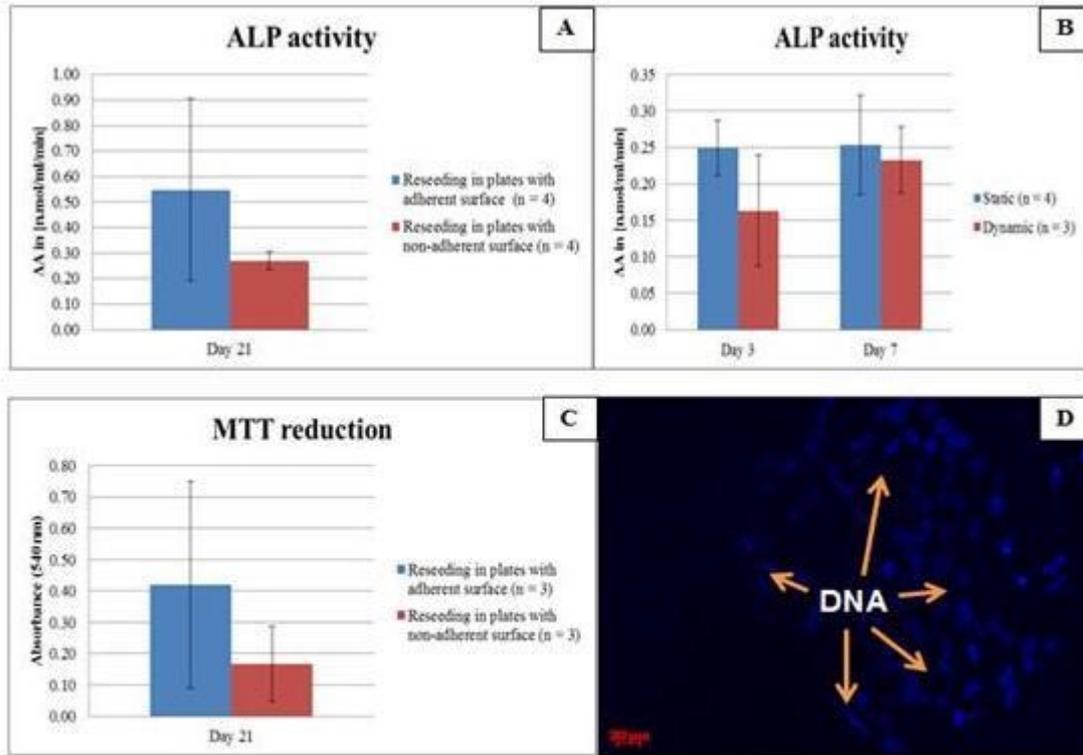


Figure 3: ALP activity of HOS cells reseeded at  $5 \times 10^5$  cells per  $\text{cm}^2$  onto bone in A) adherent and non-adherent surface for 3 weeks, B) static and dynamic environment on day 3 and day 7. C) MTT reduction of HOS cells growing on bone seeded at  $5 \times 10^5$  cells per  $\text{cm}^2$  in adherent and non-adherent surface for 3 weeks. Results are the mean  $\pm$  SEM.  $p > 0.05$  for all values by unpaired student's t-test. D) Bone samples were stained in  $400 \mu\text{l}$  of  $0.6 \text{ nM}$  DAPI on bone pieces on an adherent surface after 21 days.

Figure 3

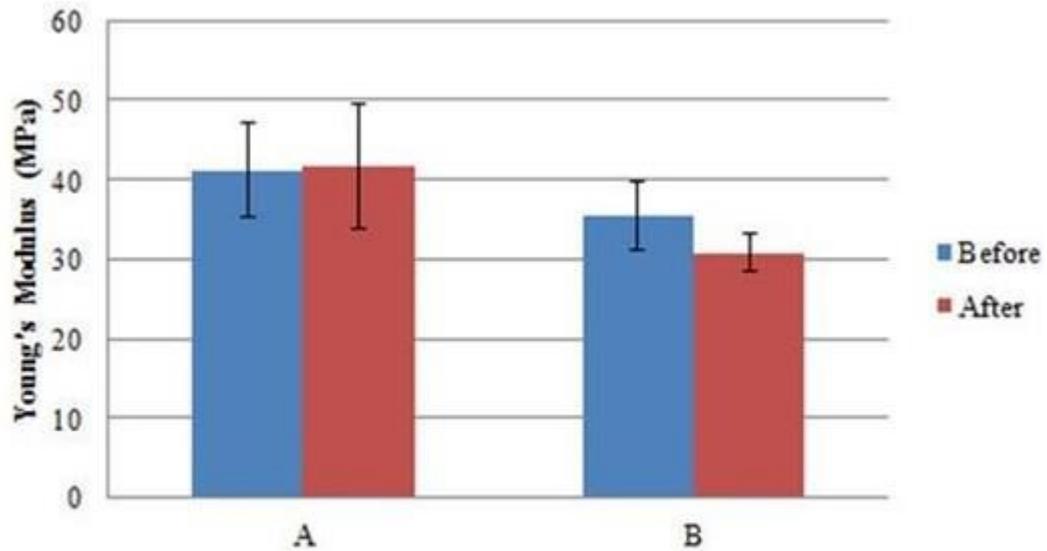


Figure 2: Young's modulus before and after six cycles of incubations with protocol A and B. A – 10mM Tris, 1mMEDTA, 0.1% v/v Triton X-100; and B – method A plus 0.5% w/v trypsin. Results are the mean  $\pm$  SEM of  $n = 6$ .  $p > 0.05$  compared with the control (ANOVA for repeated measures).

Figure 2

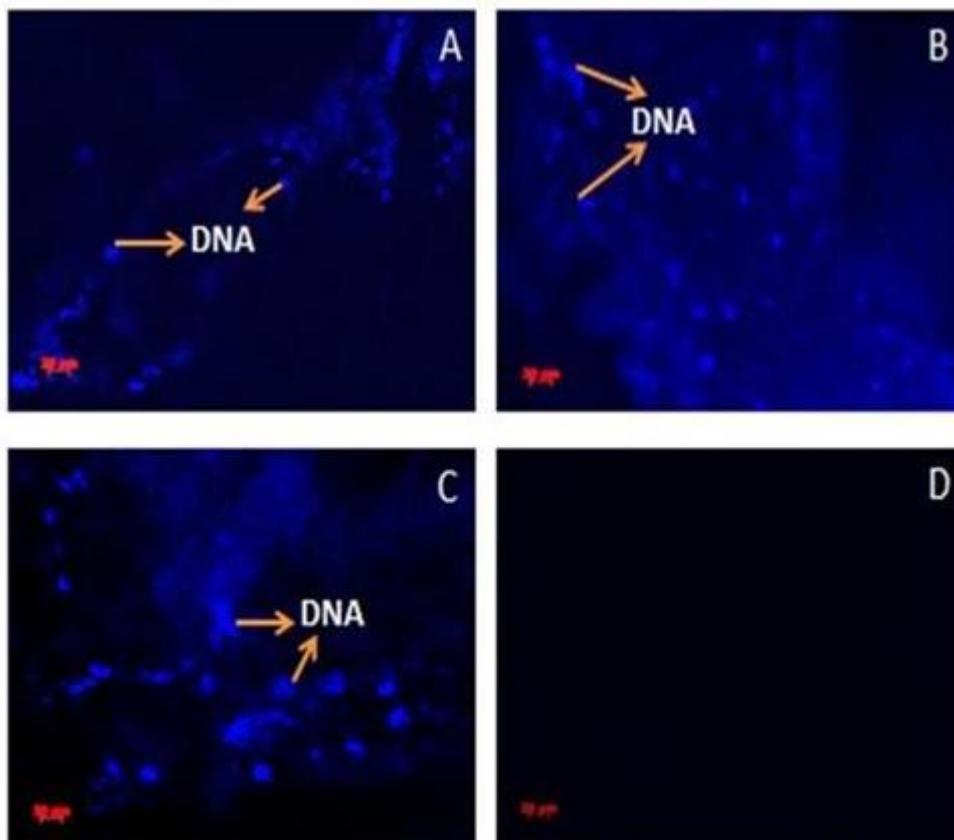


Figure 1: Bone sample tested in 0.6 nM DAPI agent A) before any treatment. B) after incubation with protocol A and B for 10 minutes. C) after overnight incubation with protocol A. D) after incubation with protocol B overnight. Protocol A – 10mM Tris, 1mMEDTA, 0.1% v/v Triton X-100; and B – method A plus 0.5% w/v trypsin. All images were taken at the surface.