Figure Legends

Figure 1: 3D experimental set up, showing the 3D porous PLGA scaffold and bioreactors used for the mechanical stimulation of the MG63.

Figure 2: Fluorescently labelling collagen through the introduction of azide-L-proline and subsequent detection utilising a commercially available, fluorescent Click-IT Alex Fluor 488 DIBO Alkyne.

Figure 3: Modification of collagen in 2D MG63 cell cultures through the addition of 36 µg/mL of azide-L-proline and the subsequent detection utilising 10 mM of Click-IT Alex Fluor 488 DIBO Alkyne in serum free media. Little background was shown in the groups where azide-L-proline was not added to the cells (top row), whereas fluorescent staining was observed in the modified collagen groups (bottom row). (Scale bars – 200 µm)

Figure 4: Modification of cell produced collagen in 3D through the addition of 36 µg/mL of azide-L-proline using MG63 cultured on fluorescent PLGA under static conditions. Once again after the addition of 10 mM of Click-IT Alex Fluor 488 DIBO Alkyne little background staining was observed in the groups where azide-L-proline was not added to the cells (top row), whereas fluorescent staining was observed on the scaffolds where the modified proline was added to the culture (bottom row). (Scale bars – 500 µm)

Figure 5: Defining the specificity of the detection of the modified collagen through the use of (a) correlation between collagen content as defined by picrosirius red assay and the fluorescence observed from the Click-IT Alex Fluor 488 DIBO Alkyne staining, showing a positive correlation between collagen concentration and fluorescence with a Pearson's R of 0.8434 ($p < 0.001$); (b) depletion of collagen through the addition of collagenase; (c) the counterstaining of collagen type I deposition and (d) the reduction in fluorescence after treatment with SiRNA to knockdown collagen expression.

Figure 6: The real-time monitoring of cell produced collagen deposition of 3D PLGA scaffolds under static, cyclic hydrostatic pressure and flow conditions. (a) The effect of the staining protocol on cell metabolism in static cultures using Alamr Blue[TM]; (b) DNA content at day 10 after three repeated Click-IT Alex Fluor 488 DIBO Alkyne staining steps at day 3, 5 and 10; (c) collagen content within the constructs using picrosirius red assay and (d) visualisation of collagen deposition within the PLGA scaffolds at day 3,5, and 10 under the differing culture conditions. (* $P < 0.05$; n=5; scale bars – 300 μm)

Supplementary Data 1: The knockdown of collagen type I, V and X after treatment with SiRNA. This gene silencing was shown to be significant across all the collagens, with a respective decrease in expression of 88.5 ± 5.9%, 74.9 ± 5.1% and 91.0 ± 3.4% of collagen type I, V and X. (* $P < 0.05$, n=3)