We confirmed expression of eNOS and beta-catenin in both UF and DF zones by quantitative-PCR and immunostaining, as well as the interaction between eNOS and beta-catenin in both regions by proximity ligation assay. We then studied the expression of several pro-survival and anti-apoptotic genes by q-PCR in HUVEC exposed to flow for 72 hours. We observed that the expression of Bcl-2 and survivin were downregulated in UF exposed cells compared to static conditions (62% ±0.08 downregulation of Bcl-2% and 67%±0.08 downregulation of survivin, n=3 $p<0.01$ in both cases); and using specific beta-catenin/TCF-LEF inhibitors we identified survivin as an anti-apoptotic gene regulated by beta-catenin (89% ±0.03 downregulation in inhibitor treated samples compared to non-treated, n=3 $p<0.001$) in endothelial cells under flow.

We also investigated the reciprocal effects on eNOS of activation of Wnt signalling and beta-catenin in HUVECs. Using Wnt3a and LiCl, that lead to the accumulation of beta-catenin in the cytoplasm, we found that phosphorylation of eNOS at Ser1177 increased 4 fold after 2–5 min (n=3 $p<0.01$) leading to enzyme activation. Phosphorylation of eNOS at Ser633 and Ser114 was also observed both in HUVEC exposed to UF flow for 72 hour in an orbital shaker and in HUVEC stimulated by Wnt3a.

Together our results indicate that beta-catenin is a key mediator of flow-induced anti-apoptotic effects, both through transcriptional regulation and through activation of eNOS phosphorylation in endothelial cells.

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173 INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 2 (IGFBP2): A POSITIVE REGULATOR OF ANGIOGENESIS?

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Introduction The insulin-like growth factor binding protein 2 (IGFBP2) has been implicated in the regulation of insulin-like growth factor (IGF) activity in most tissue and organs. IGFBP2 has, however, been reported to have additional intrinsic, IGF independent properties. Low circulating IGFBP2 levels are associated with obesity in humans. High levels of IGFBP2 on the other hand are linked to increased tumour angiogenesis in humans. In this setting increased angiogenesis has been suggested to be caused by indirect rather than direct modulation of endothelial cells. Here we tested the hypothesis that IGFBP2 is able to modulate endothelial cell function directly.

Basic methods and Results: Using immunoblotting, we show that acute stimulation of human umbilical vein endothelial cells (HUVEC) with 15 nM IGFBP2 lead to an increase in phosphorylation of Akt/PKB, an important regulator of endothelial cell function. Data obtained from an *in vitro* model of sprouting angiogenesis suggests that stimulation of HUVEC with IGFBP2 induced endothelial cell sprouting. Mice overexpressing human IGFBP2 showed increased tip cell formation and vascular density in the mouse retina model of developmental angiogenesis.

Conclusions Here we present data from *in vitro* and *in vivo* models of angiogenesis supporting our hypothesis that IGFBP-2 is able to directly modulate endothelial cell function.

174 COMPARISON OF THE MECHANICAL PERFORMANCE OF POLYMERIC AND METALLIC SCAFFOLDS – TESTING AND MODELLING

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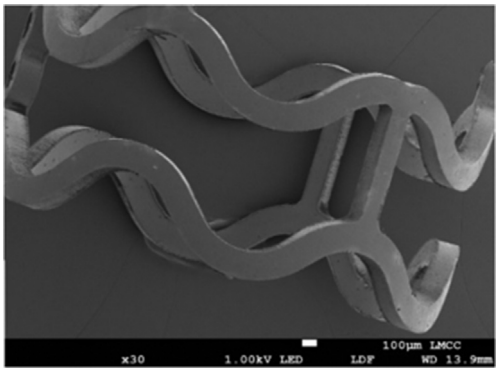
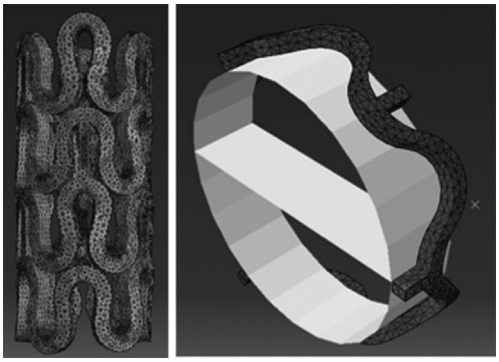
Percutaneous coronary intervention is a standard procedure to resolve blockages within artery, which involves the implantation of stents to maintain vessel patency. Currently, bioresorbable scaffolds (BRSs) are in the process of replacing the metallic permanent predecessor (drug eluting stents) commonly used in stenting. BRSs are commonly made of poly (L) Lactide (PLLA), an aliphatic polyester which is biodegradable and biocompatible with a wide range of medical applications. The performance of these scaffolds is not well defined in comparison to their metallic counterparts.



Abstract 174 Figure 1 SEM image of a polymeric scaffold.

The aim of this project is to assess the mechanical performance of PLLA scaffolds (Figure 1), with a direct comparison to that of metallic stents. This will be achieved through mechanical testing of structural rings at different load rates and ranges. Scaffolds will also be characterised using nano/micro indentation. The results will be used to support computational work for predicting the behaviour of both stents during crimping and expansion (Figure 2).



Abstract 174 Figure 2 Illustration of computational assessments of BRS scaffold performance (ring test and crimping).

Figure 3, Nanoindentation data on BRS.
Figure 4, AFM data on BRS.

Preliminary work indicates that it is possible to assess the local mechanical properties of a stent by atomic force microscopy and nano-indentation through evaluation of the unloading curves (figures 3 and 4). Further work would incorporate assessing the performance of the polymer scaffolds at different degradation time points to ascertain that vessel patency is achieved before complete degradation of BRSs.

Results obtained here will help gain a better understanding of local and global mechanical properties of BRSs and enable further research and development of the scaffolds.

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175 THE EFFECTS OF TBQ ON CARDIAC INTRACELLULAR ATP LEVELS; ROLE OF OXIDATIVE PHOSPHORYLATION AND OXIDATIVE STRESS

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In a recent study, 2,5-Di-(tert-butyl)−1,4-benzohydroquinone (TBQ) produced a concentration dependent and fully reversible inhibition of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) in rat ventricular myocytes[1]. While TBQ is a potentially useful research tool to study SERCA inhibition in cardiac cells, many additional effects were observed including production of an outward current consistent with activation of an ATP dependent potassium channel. The current study aims to determine the mechanisms underlying these effects.

Rat and sheep ventricular myocytes were isolated by enzymatic digestion. Intracellular ATP levels were measured using a ViaLight Plus Cell Proliferation Kit (Lonza). Mitochondrial oxygen consumption and levels of hydrogen peroxide were measured using an Oxygraph-2k high resolution respirometer (Oroboros Instruments).

In rat and sheep ventricular myocytes, TBQ produced a concentration dependent decrease of intracellular ATP where 100 Î¼M TBQ decreased ATP levels to approximately 60% of control. Removal of glucose from the experimental solutions had no effect on the magnitude of effect. In rat homogenates, TBQ produced a concentration dependent decrease of mitochondrial oxygen uptake, with 100 Î¼M TBQ decreasing rate to 85% of control. TBQ increased levels of hydrogen peroxide, however catalase, did not attenuate TBQs effect on mitochondrial oxygen uptake.

The current findings suggest TBQ decreases intracellular ATP, a phenomenon which may account for many of the effects observed previously, including activation of an ATP dependent potassium channel. The reduction in ATP appears to be associated with an effect on oxidative phosphorylation rather than glycolysis. While TBQ is associated with an increase in hydrogen peroxide, which *may* increase oxidative stress, the experiments carried out with catalase suggest that this does not contribute to TBQs effect on mitochondrial function.

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176 SEROTONIN RECEPTOR 2B (5-HT2B) MODULATES CARDIOMYOCYTE PROLIFERATION BY REGULATING THE HIPPO PATHWAY

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Heart failure is one of the leading causes of death worldwide. In part, this is due to the inadequate regenerative capacity of cardiomyocytes post-injury. Modulation of the Hippo signalling pathway in mice has been shown to enhance cardiomyocyte proliferation and improve survival in a myocardial infarction model. While the discovery of the Hippo pathway and its function as a master regulator of cell proliferation has led to greater understanding of its core components such as Yes-associated protein (YAP), the upstream signals that regulate the Hippo pathway have remained elusive. This study was aimed to identify novel upstream regulators of the Hippo pathway in cardiomyocytes that could be targeted pharmacologically to induce regeneration.

We performed a targeted RNAi screen in H9c2 cardiomyoblast cell line using adenovirus-mediated luciferase reporter system to detect the activity of YAP, the major effector of the Hippo pathway. Using this system, 5-hydroxytryptamine receptor 2B ($5\text{-}HT_{2B}$) was identified as a potential regulator of the Hippo pathway. Serotonin-mediated $5\text{-}HT_{2B}$ has previously been shown to play a significant role in cardiac development during embryogenesis; however, a link between $5\text{-}HT_{2B}$ and the Hippo pathway has not yet been documented.

An *in vitro* model was subsequently established by overexpressing $5\text{-}HT_{2B}$ in primary neonatal rat cardiomyocytes (NRCMs) using an adenoviral system. The activities of different components of the Hippo pathway were investigated with an emphasis on YAP. Immunofluorescence microscopy was utilised to quantify cardiomyocyte proliferation and survival.

Using this system, we found that overexpression of $5\text{-}HT_{2B}$ in cardiomyocytes enhanced YAP activity by 12 folds compared to the control group as indicated by YAP-luciferase assay. In keeping with this, we observed an increase in YAP nuclear translocation following $5\text{-}HT_{2B}$ overexpression, indicating YAP activation. Mechanistically, we found that $5\text{-}HT_{2B}$ expression reduced Large tumour suppressor (LATS) phosphorylation, eventually leading to YAP activation. Since YAP is known to mediate cell proliferation we analysed proliferation rate in cardiomyocytes overexpressing $5\text{-}HT_{2B}$. We found that cell proliferation was increased by 39.7% compared with control cells as indicated by EdU incorporation assay.

In conclusion, our findings have identified $5\text{-}HT_{2B}$ as a novel upstream regulator of the Hippo pathway in cardiomyocytes. We also observed that in cardiomyocytes, $5\text{-}HT_{2B}$ is a potent stimulator of YAP activity and cell proliferation. Since $5\text{-}HT_{2B}$ is a membrane receptor that can be targeted pharmacologically, this finding may provide new insight for the development of a new approach to induce cardiomyocyte regeneration.