MMP-9 ACTIVITY IS INCREASED IN THE FIBROBLASTS BUT NOT MYOBLASTS IN RESPONSE TO GLYCOPROTEIN COATING

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Introduction
Tenderness is an important meat quality and a common cause of unacceptability of meat purchases is toughness (Jeremiah, 1982). Both myofibrillar and intramuscular connective tissue (IMCT) components of meat contribute to cooked meat toughness. Whilst is generally accepted that meat tenderisation post-mortem is primarily a result of proteolysis of myofibrils by endogenous calpain enzymes (Koohmaraie, 1991), both myofibrillar and IMCT components are turned over during muscle growth. A large family of at least 26 structurally-related zinc metalloproteinases (MMP's) is primarily responsible for connective tissue catabolism (Nagase *et al.*, 2006). Skeletal muscles contain many enzymes of the MMP family and their inhibitors (Balcerzak *et al.*, 2001). There is good evidence that MMP expression in skeletal muscle is influenced by nutritional status and mechanical stimulation (e.g. exercise). IMCT is structurally and functionally complex; it is divided in to perimysium and endomysium, both with different collagen and proteoglycan components, and different structural organization. It is not clear which cell types within muscle are responsible for the maintenance and turnover of which IMCT components. As part of a basic cell culture study of MMP expression by a variety of cell types found in muscle, the current research was conducted to examine the activity of MMPs secreted by the cultured fibroblasts and myoblasts and how activity is modulated by the IMCT components that the cell types are interacting with. We studied activity of two important MMP enzymes; MMP-9 (also called gelatinase B) is most active in hydrolysing gelatin, native type IV collagen and elastin (Murphy and Crabbe, 1995) and MMP-3 (stromelysin-1) is capable of degrading proteoglycans, gelatin, type IV collagen, laminin, fibronectin, and type IX collagen (Okada *et al.*, 1989).

Materials and Methods
Fibroblasts (Rat 2, R2) and myoblasts (C2C12) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum. Substrates diluted in PBS were coated to the tissue culture plates by incubating overnight at 4°C. The plates were air-dried before use. Equal number of cells was loaded into the coated and the non-coated plates for each substrate coating experiment. The cells were then growth at 37°C in 5% CO_2. After 24 hours, the culture medium from each plate was collected and freeze-dried to 3:1 concentrated and the cells in the plate were counted. The concentrated culture medium was used to test the MMP activity.
The MMP-9 and MMP-3 activities in the culture medium were determined by gelatin or casein zymography, respectively. Gelatin zymography was carried out with minor modification of the method of Kleiner and Stetler-Stevenson (1994). Casein zymography was performed according to .t(he method described by Fernandez-Resa *et al.*, (1995). Data were analysed by one-way ANOVA using an SPSS general linear model program. Means were separated by Fisher's protected least significant difference (LSD) test.

Results and Discussion
The rate of C2C12 myoblast differentiation into myotubes is known to be affected by the type of extracellular matrix component they are in contact with (Lawson and Purslow, 2001) To look at effects of interactions on the activity of MMPs expressed from the cells, we chose collagen I fibronectin, laminin as test IMCT components and used bovine serum albumin (BSA) as the negative control.
Figure 1 shows a gelatin zymogram gel showing MMP-9 activity in fibroblasts and myoblasts grown on laminin. (MMP-9 standard; R2 fibroblasts on no coating; L+R2 fibroblasts on laminin coating, C2 myoblasts on no-coating, and L+C2 myoblasts on laminin coating in lane 1 to lane 5, respectively. Generally, fibroblast show more MMP activity than myoblasts. Laminin coating did not modify MMP-9 activity in myoblasts (no difference in band densities between the Lane 4 and Lane 5), but, laminin coating increased MMP-9 activity in fibroblasts (increased band density in the Lane 3 as compared to that in Lane 2).
Figure 2 shows that, compared to control (non-coated cultures) interactions with glycoprotein (fibronectin (FN), laminin) substrates increased mean MMP-9 activity (n=4 cultures) in the fibroblasts as compared with that of in the myoblasts ($p<0.05$). However, the proteins collagen I and BSA have no such effect. Myoblasts are likely to interact with laminin in the basement membrane far more than Collagen I in the intracellular matrix
In contrast with MMP-9, MMP-3 activity in either the fibroblasts or the myoblasts as examined by casein zymography was not influenced by coating any of the substrates tested (Data not shown).

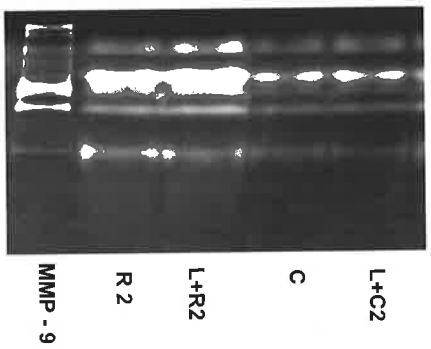
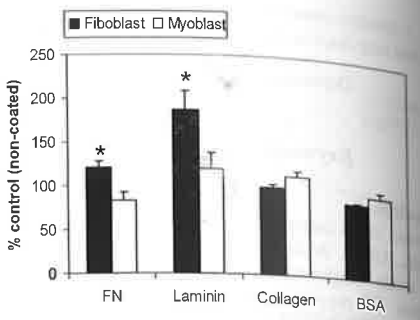


Figure 1.

Figure 2.



Conclusions

This research demonstrates that MMP activity expression from fibroblasts and myoblasts in culture is different. Interactions between cells and IMCT components also interactively influenced MMP-9 activity. Laminin coating increased MMP-9 activity in fibroblasts but not in myoblasts. MMP-9 activity increased in fibroblasts in response to glycoprotein coating as compared with that in myoblasts. These interactions may be important in controlling the expression of MMPs by fibroblasts in the endomysium and perimysium during muscle growth and MCT turnover.

References

Balcerzak, D. *et al.*, (2001) *Journal of Animal Science*, 79, 99-107.
Fernandez-Resa, P. *et al.*, (1995) *Anal. Biochem*, 224, 434-435.
Jeremiah, L.E. (1982) *Journal of Consumer Studies and Home Economics*, 6,137-154.
Koohmaraie, M *et al.*, (1991). *Journal of Animal Science*, 69, 617-624.
Kleiner, D.E. and Stetler-Stevenson, W.G. (1994) *Anal. Biochem*, 218,325-9.
Lawson, M.A. and Purslow, P.P. (2001) *Br. J. Poultry Science*, 42, 315-320.
Murphy, G. and Crabbe, T. (1995) *Methods Enzymol*, 248:470-84.
Nagase, H. *et al.*, (2006) *Cardiovasc. Res.* 69, 562-573.
Okada, Y, *et al.*, (1989) *FEBS Lett*, 244,473-6.