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SCIENTIFIC SECTION

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Muscling in on malocclusions: Current concepts on the role of muscles in the aetiology and treatment of malocclusion

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The British Orthodontic Society invites outstanding contributors from the field of Orthodontics to give the guest lecture in memory of George Northcroft. In 2005 the guest lecturer was Professor Nigel Hunt. The article that follows was presented as the Northcroft Memorial Lecture 2005 at the World Orthodontic Congress, Paris.

Invited paper

Introduction

As clinicians the need to have a clear and in-depth understanding of the mechanisms of both normal and abnormal facial growth should be apparent to all. When abnormalities of growth are detected, modern technology facilitates the interception of developing malocclusions, for example, through the use of myo-functional appliances or distraction osteogenesis techniques. Similarly, advances in surgical techniques and methods of fixation enable us to correct a variety of established malocclusions, primarily of skeletal origin, through the use of a combination of orthodontics and orthognathic surgery. Of all the problems that confronts us it is abnormalities in the vertical dimension, whether they be in the growing child or the adult, which still present the greatest difficulties both in treatment itself, as well as maintenance of the treatment outcome.

For normal, vertical facial growth and development, growth in the anterior part of the face must equal that occurring in the posterior part of the face. Should this not occur, then a relative growth rotation of the mandible can develop. For example, should growth in the posterior part of the face exceed that occurring anteriorly, then the net effect is an anterior, forward closing rotation of the mandible producing the typical short face deformity and deep overbite associated with the short face syndrome.[1] At the opposite extreme where growth in the posterior part of the face may be severely reduced in comparison to that occurring anteriorly, an opening or clockwise rotation of the mandible is evident with the net effect being an excessive anterior facial height and frequently an anterior open bite, associated with a long face deformity.[2]

For generations, both clinicians and scientists have argued as to the respective contribution of genetics and so called environmental factors in influencing ultimate facial form and associated malocclusion. Of all the possible environmental influences, it is not surprising that bearing in mind the origins and insertions of the muscles of mastication, and in particular the masseter and medial pterygoid muscle, that the question has arisen as to whether or not abnormalities in the structure and function of the muscular pterygomasseteric sling could, in any way, influence vertical development in the posterior part of the face. Furthermore, if treatment interventions necessitate a change in function of the muscles that support the mandible, do the adaptive capability of these muscles in any way influence the stability of the treatment outcome?

This essay discusses some of the research that has been undertaken in my department over the past 10 years and has tried to provide evidence to address these issues. I will therefore divide this essay into three sections, and discuss;

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- the role of muscles in the aetiology of vertical facial deformity;
- the influence of muscles on treatment outcome;
- potential developments with regard to the manipulation of muscle.

The role of muscles in the aetiology of vertical facial deformity

What evidence do we have that muscle structure and function is related to facial form? Human clinical studies of muscle function have mainly revolved around the measurement of either occlusal force, or muscle volume and area in relation to differing facial morphologies. It is now evident that patients with a short vertical dimension are capable of producing occlusal forces far in excess of those recorded from patients with normal vertical facial form. Conversely, individuals with long face deformities produce very weak occlusal forces as measured either during swallowing, chewing or maximum clenching.[3] With regard to muscle volume, 3-D ultrasound studies have shown that patients with large posterior face heights and low maxillary mandibular plane angles are associated with large masticatory muscles.[4]

The question remains, however, as to which comes first. Are these functional differences a reflection of a primary inherent abnormality of muscle structure or are the recordings merely a reflection of an adaptive response to the relative efficiency of the muscle system? It is incumbent upon us as clinicians to know the answer to this question before we can apply appropriate treatment modalities and to achieve stable results. It is therefore important that we have a complete understanding of skeletal muscle structure before we can try to address the conundrum as to whether or not muscles are a prime aetiological factor in the development of aberrant vertical facial form.

Muscle consists of a contractile apparatus (the muscle fibres) sited in an intimate relationship with the extracellular matrix; whether it be around individual muscle fibres, groups of fibres or surrounding the entire muscle. Scattered throughout the muscle structure, but in intimate relationship with the basal lamina are muscle-derived stem cells (satellite cells). Using very basic histochemical staining techniques it has been shown that the muscles of mastication, in accordance with other skeletal muscles of the body consist of a mixture of muscle fibre types, and which are broadly categorized as being either type I or type II fibres. These fibres differ in both their speed of contraction and in their metabolic activity. Type I fibres are slow acting, but fatigue resistant and it is believed that they are responsible for such activity as generation of posture. On the other hand, type II fibres are fast acting, but fatigue easily and it is understood that these fibres produce maximum force of short duration such as that occurring during clenching of teeth. The typical picture, therefore, seen in a patient with a short face deformity is such that the type II fibres are present in greater numbers than those seen in patients with normal facial dimensions. It is uncommon, however, to see appreciable variation in fibre size in either the type I or the type II fibres. In contrast, patients with long face deformity show not only a reduced number of type II fibres, but these fibres also appear smaller in size in comparison to the normal size type I fibres. Bearing in mind the differing physiological roles of the fibre types and, in particular, that the type II fibres are the major contributor to maximum force, these observations concur with the clinical observations of measurements of occlusal force (Figure 1).

Although many proteins, including Troponin and Tropomyosin B, are involved in muscle contraction, it is the interaction of the rod-like myosin molecules with the globular-like actin molecules which is fundamental to the process. The myosin molecules involved in this contractile arrangement are the heavy molecular weight proteins referred to as the myosin heavy chain proteins (MHCs). In contrast to limb muscles, the adult masseter muscle is shown to contain at least five myosin heavy chain isoforms ranging from the type I myosin heavy chain gene (MYH 7), which is primarily responsible for the slow phenotype, and the fast phenotype components represented as type IIX and IIA heavy chain proteins (MYH 1 and MYH 2 genes, respectively). In addition, there are at least two more isoforms not normally found in adult limb skeletal muscles, but which can be considered as normal components of the masticatory muscles. These include the alpha cardiac myosin heavy chains (MYH 6 gene), normally found in heart muscle, and developmental myosin heavy chains which normally disappear from other skeletal muscles shortly after birth (MYH 3 and 8 genes).[5] Comparing the myosin heavy chain isoform expression of patients with long face deformity as opposed to those with normal vertical dimensions, it is apparent that there are at least two variations from this typical picture. First, the fast isoform is markedly reduced in its presence and, secondly, the proportion of developmental isoforms is frequently increased albeit with large individual variation (Figure 2).

Whether or not these changes in myosin heavy chain expression are an inherent genetic abnormality or whether they are merely a response to altered function

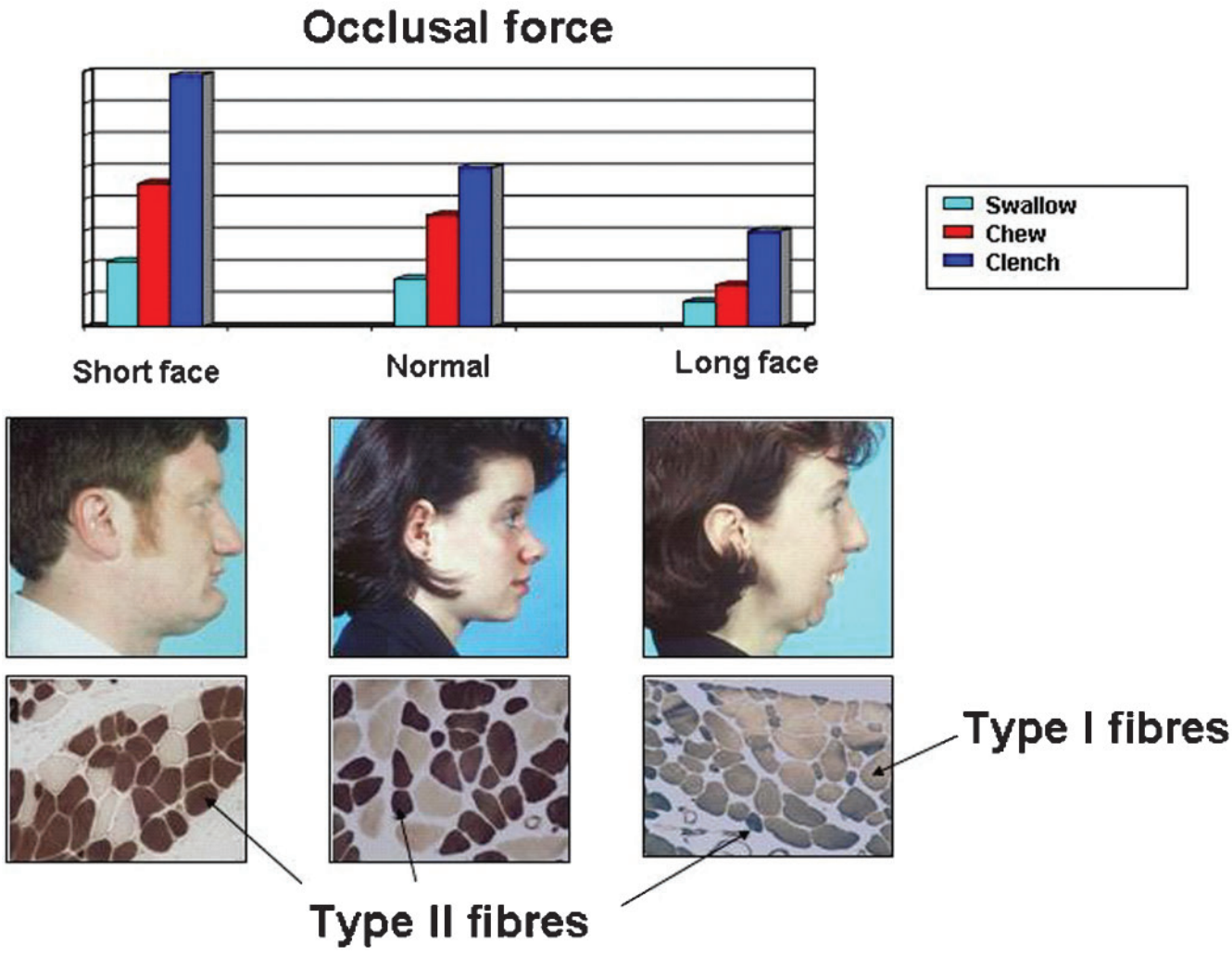


Figure 1 Patients with a short face have a predominance of fast type II fibres and are capable of generating higher occlusal forces than those patients with normal vertical dimensions. Conversely, long face patients have few, small type II fibres and generate relatively weak occlusal forces

can be tested by the experimental manipulation of skeletal muscles. Typically, if an adult limb muscle is overloaded, for example, through repeated contraction and relaxation, then the response is one of fibre hypertrophy affecting both the slow and fast fibres, but with an overall increased expression of slow isoforms. With regard to masticatory muscle, the differences in fibre size evident in patients with increased vertical facial deformities are a reduction in the size of the fast fibres only. Although there may not be an increased expression of slow isoforms *per se*, for a given volume of muscle, because the fast isoforms are smaller in size and number, there may be a *relative* increase in expression of the slow isoforms. At the opposite extreme, if a muscle is unloaded, for example by applying plaster cast fixation to a limb, then the adaptive changes seen are typically those of fibre atrophy. Again, both slow and fast fibres are affected but with an overall increased expression of fast isoforms. Clearly, this does not fit neatly with the pattern seen in the masseter muscles of patients with long face deformities. Therefore, it would appear that the changes seen are not typical of a limb skeletal muscle, which has been subject to either overwork or disuse and on this evidence alone, suggests that the variation seen in the fast/slow fibres may not be solely adaptive in nature. This analogy, however, makes the basic assumption that the masticatory muscles adapt and respond in the same manner as limb muscle. Indeed, the evidence as to whether or not this is true is somewhat equivocal, and it is important to bear in mind that the masticatory muscle differs from limb muscle in several ways, including being of a different embryological origin, as well as being innervated by cranial, rather than spinal nerves.

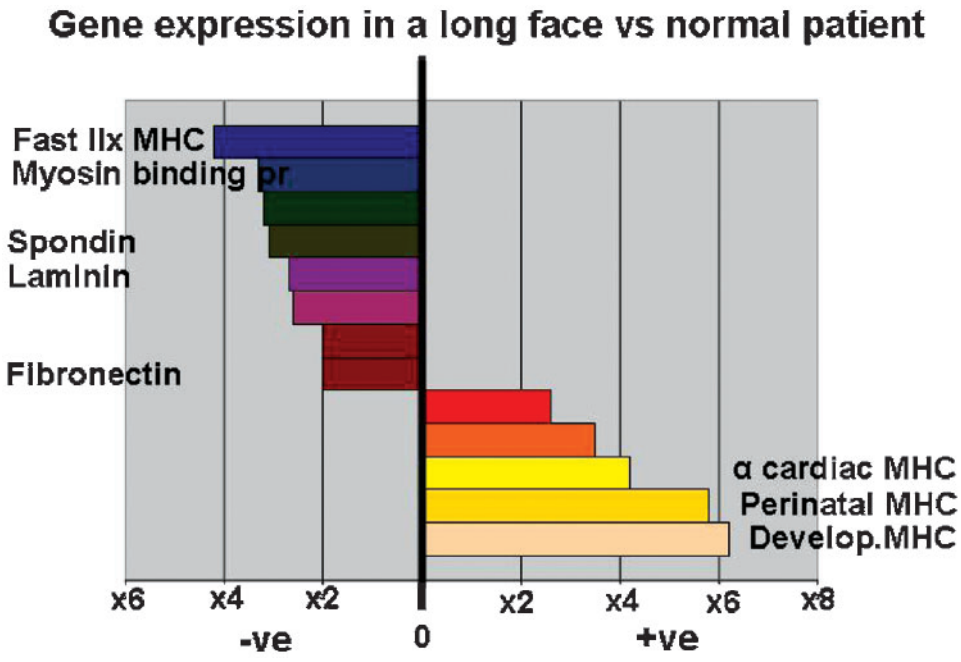


Figure 2 A selected printout of the relative gene expression typical of a long face (using a normal facial form as the comparator)

The second change noted in patients with long face deformity with regard to myosin heavy chain expression is the increased presence of developmental heavy chain myosin isoforms. Again, large individual variation is evident. It has been postulated that such developmental isoforms may form part of the adaptive process whereby these rudimentary isoforms can adapt into either slow or fast isoforms depending upon the functional requirements. With regard to masticatory muscles, it has been postulated that this could reflect the quality of the occlusion and the efficiency of the occlusal forces. There is some evidence to support this as indicated by a study which compared the presence of developmental myosin heavy chains in relation to the number of occlusal contacts seen in a group of patients with a variety of both normal and abnormal facial form.[6] There is an inverse relationship present such that the greater the number of occlusal contacts, the lower the presence of developmental myosin heavy chains (Figure 3). One explanation for this could be that in patients with a poor occlusion and few occlusal contacts, the muscles are repeatedly undergoing micro-trauma, and are regenerating and adapting in order to try to produce a more efficient functioning muscle-occlusal system.

Gene expression studies

Following the publication of the human genome it has now become possible to examine the total gene expression in a particular body tissue using micro-array technology, rather than multiple investigations of single structural components. RNA extracted from a muscle biopsy can be amplified through a process of reverse transcription, and following fluorescent labelling can be hybridized to the DNA on a microchip. The varying levels of fluorescence emitted from the individual array gives the relative expression of a particular gene sequence. This can then be read by a computer to give the relative gene expression of a tissue from one subject compared to another. One example of this is shown in Figure 2 where the selected gene expression from a long face patient is compared with that from a patient with normal facial form. In this example, it can be seen that the genes responsible for the IIX myosin heavy chain protein, as well as the myosin binding proteins and alpha 6 integrin expression in addition to other components of the extra-cellular matrix such as fibronectin, are shown to demonstrate between and a two- and four-fold reduction in gene expression compared with a patient with normal facial form. Conversely, there is between a four- and six-fold increase in other myosin heavy chain components; for example, the alpha-cardiac and developmental isoforms. Again, it is emphasized that there is large individual variation between patients with a specific facial form.

Therefore, to return to the original question as to whether the muscles of mastication could have a prime role in the aetiology of malocclusion it would appear that although gene expression differences exist in both the contractile and extra-cellular matrix components of

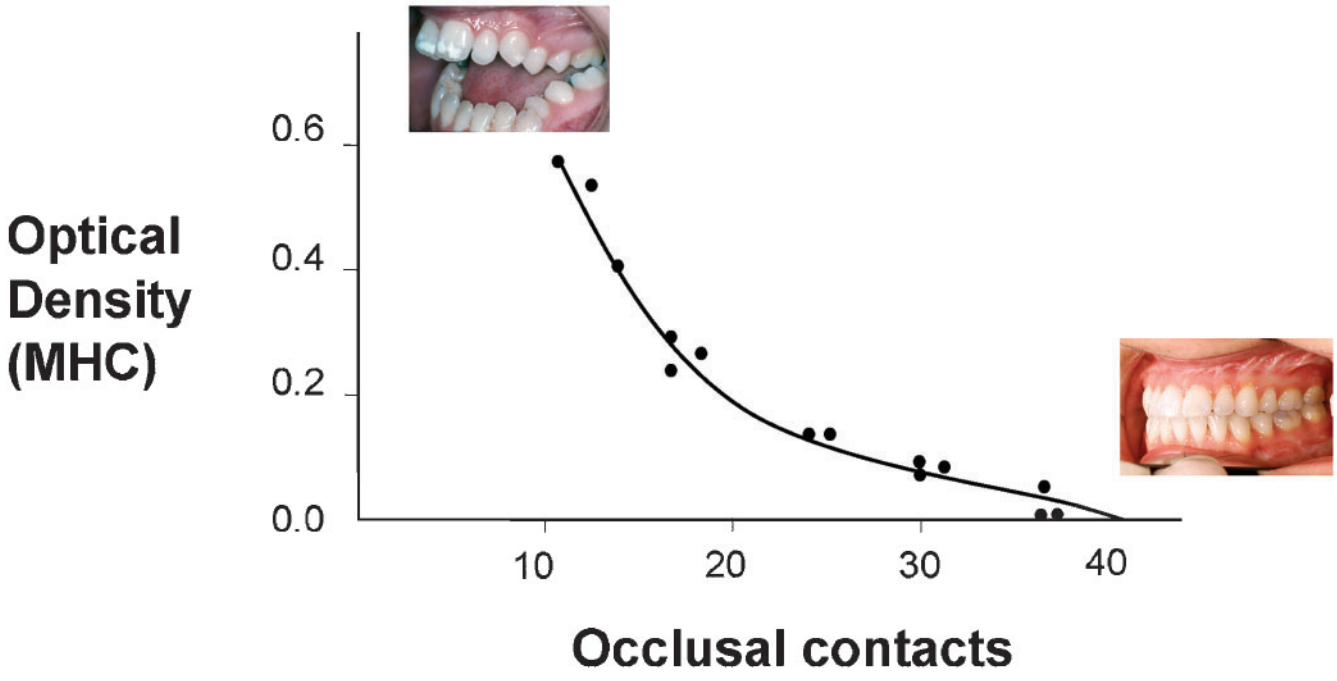


Figure 3 The presence of developmental MHC proteins shows an inverse relationship with the quality of the occlusion as measured in terms of number of occlusal contacts

masseter muscle in some patients with increased vertical facial deformity, there is large individual variation. It is suggested that the majority of these changes are probably of an adaptive nature in response to changing functional demands. However, some patients have such diverse variation that the possibility that they may have inherent muscle pathology still cannot be totally ignored. The next step in solving the original conundrum is to examine the gene sequences in those genes showing expression differences with regard to the variations in facial form. This work is still ongoing.

The role of muscles in response to treatment

Many forms of interceptive treatment, whether they be purely orthodontic in nature or in combination with surgery, bring about changes in the muscles of mastication with regard to one or more of the following changes:

- in muscle fibre orientation;
- in the functioning length of fibres;
- in muscle structure;
- in muscle phenotype.

Successful treatment requires both reorganization in the connective tissue and regeneration of muscle fibres. Reorganization of connective tissue is an extremely complex process involving muscle derived stem cells (satellite cells), extra-cellular matrix molecules and receptors for the extra-cellular matrix (for example integrins). Remodelling of the extra-cellular matrix is mediated by a family of enzymes known as matrix metalloproteinases (MMPs).[7,8] MMP2 is expressed during the regeneration of new myofibres and is a known mechano-responsive gene. A knowledge of how muscles respond to clinical interventions is pivotal to treatment success and can influence the way in which a particular treatment modality is applied. Functional appliances, for example, can be either fixed or removable, can be constructed to varying degrees of vertical opening, and there are protagonists and antagonists for both gradual versus one-step activation of the appliances. Similarly, distraction osteogenesis is considered by many to be preferable to orthognathic surgery in specific cases because it induces a gradual as opposed to a one-step activation believed to be more physiologically appropriate for bone and, possibly, muscle adaptation.

One way of assessing how muscle responds to stretch is to study the fate of developing muscle cells when grown in a three-dimensional muscle cell culture. Using MMP2 production as a marker of muscle adaptation in response to stretch, it has been shown that the muscle response is greater with a continuous stretch regime in contrast to an intermittent or cyclical stretch process. Furthermore, the muscle response is directly proportional to the amplitude

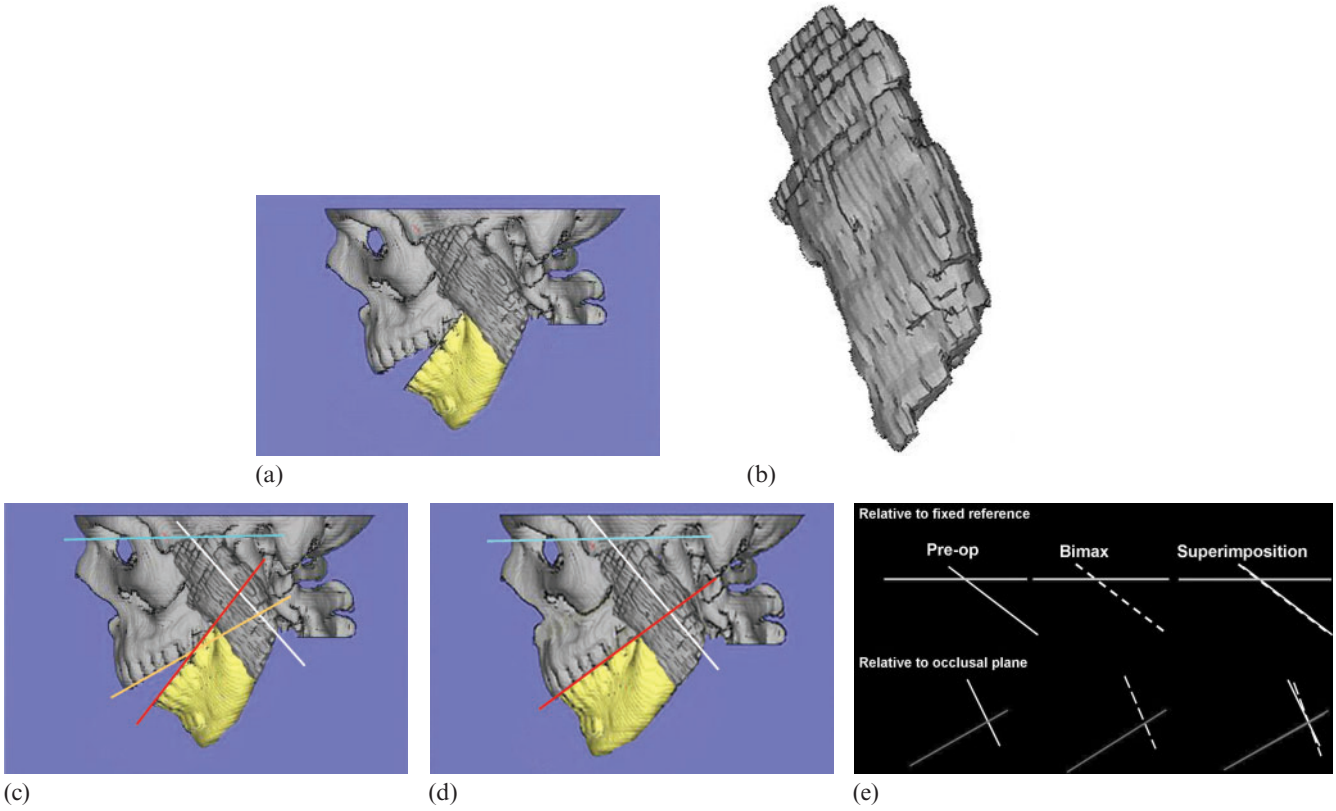
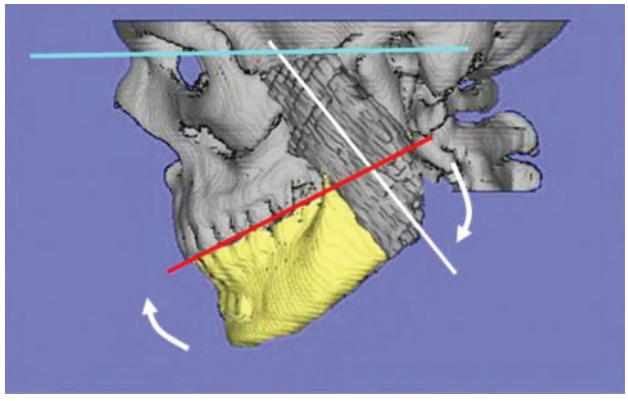


Figure 4 (a,b) 3-D MRI showing detail of masseter muscle fibre bundle orientation. (c–e) Favourable change in muscle length and fibre orientation following maxillary impaction and mandibular advancement surgery for closure of anterior open bite

of the stretch.[9] Clinically, this would suggest that posturing the mandible both horizontally and vertically, to an extent that exceeds the freeway space, means the greater the likelihood of an adaptive muscle response, rather than one that is of limited vertical opening. Furthermore, a fixed form of functional appliance, for example, the Herbst appliance, would be expected to bring about a more responsive change than a removable appliance due to the likely intermittent wear of the latter. These results would also lend some weight to the suggestion that incorporation of a screw into the appliance, whereby the activation can be gradually increased, is more likely to lead to adaptation than one in which there is a one-step activation. Similarly, these results suggest that a more gradual protraction of the mandible, as may occur in adult patients for example as part of distraction osteogenesis, is more likely to be stable through gradual muscle adaptation than a one-off insult as a consequence of orthognathic surgical advancement of the mandible.

With regard to orthognathic surgery the golden rule is that surgery must not stretch the pterygo-masseteric sling, otherwise relapse is likely to occur. This is predominantly through the speed of insult to the muscle in relation to the timing of the muscle adaptive process. The consequence is either an immediate reversion back to the original functioning length of the muscle and return of the bony fragments back to their original pre-surgical position, and/or migration of the muscle attachment along the surface of the bone, thereby leading to an area of bone denuded of muscle force, which ultimately leads to resorption of the bony muscular processes. One way in which this can be studied more closely is through refinements in protocols for 3-D magnetic resonance imaging of the face and jaws. Increasing the resolution of the tomographic cuts to approximately 1.0 mm has led to a resolution which facilitates the identification of not only the origins and insertions of the muscles of mastication but even the orientation of individual muscle fibre bundles (Figure 4a,b). It is therefore possible to study the changes in muscle fibre orientation in relation to landmarks such as the functional occlusal plane and also those landmarks unaffected by surgery, for example, the cranial base (Figure 4c,d). Ideally, as mentioned, surgery to correct an increased vertical facial deformity should involve posterior maxillary impaction together with a mandibular procedure where



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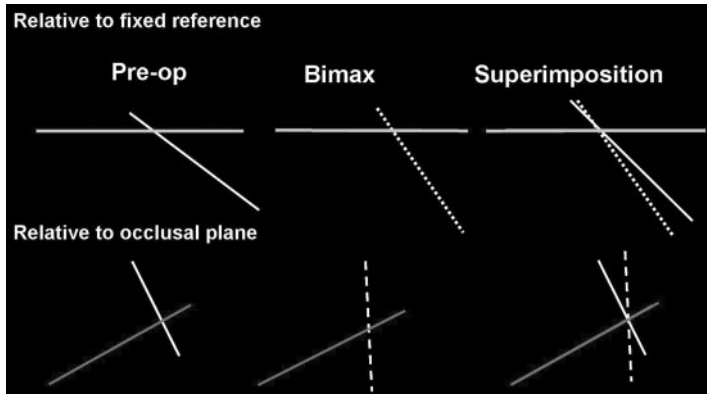
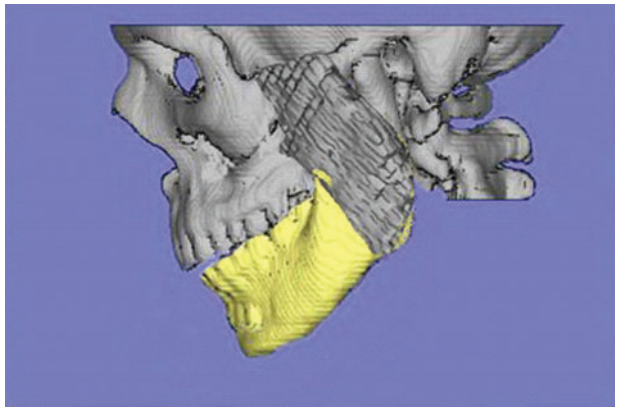


(g)

Figure 4 (f,g) Unfavourable change following insufficient posterior maxillary impaction with resultant stretch of pterygo-mandibular sling

the final outcome does not increase the posterior facial height and hence, does not stretch the pterygo-masseteric sling. As such the orientation of the muscle fibers in relation to their functioning occlusal plane remains unaltered (Figure 4e). However, if there is a failure adequately to impact the posterior part of the maxilla in such cases then there is a rotation of the mandibular segments around the premolar/first molar region resulting in a reduction of the anterior face height but an unwelcome increase in the posterior vertical dimension (Figure 4f) and, thereby, leading to an increase in the length of the pterygo-masseteric sling (Figure 4g). Furthermore, this leads to a much less efficient musculo-occlusal relationship and as such more extensive adaptation has to take place within the muscles in order to be able to accommodate the unwanted surgical change. A preliminary study has shown that at one year following surgery, where this unwanted change has occurred, there is not only a return towards the original pre-surgical bony relationships (Figure 4h), but also migration of the muscle attachment leaving an area of bone at the gonial angle, which subsequently resorbs and leads to the unwanted and unsightly hour-glass deformity of the mandibular border (Figure 4i).

The exact mechanism of muscle fibre adaptation remains somewhat controversial and, in the past, it has been postulated that a group of muscle fibres with a particular phenotype, which contains the developmental myosin heavy chains, may provide a source of 'immature' muscle fibres, which could adapt in response to differing functional needs into predominantly slow or fast fibre types. An early investigation, which followed the fate of such fibres in 12 consecutive patients who had undergone bimaxillary procedures for closure of an anterior open bite associated with a Class II long face deformity has shown that at 12 months following surgery there is a significant reduction in the presence of these developmental isoforms.[10] Bearing in mind the relationship between quality of the occlusion and prevalence of developmental myosin heavy chain isoforms described above this could merely reflect the



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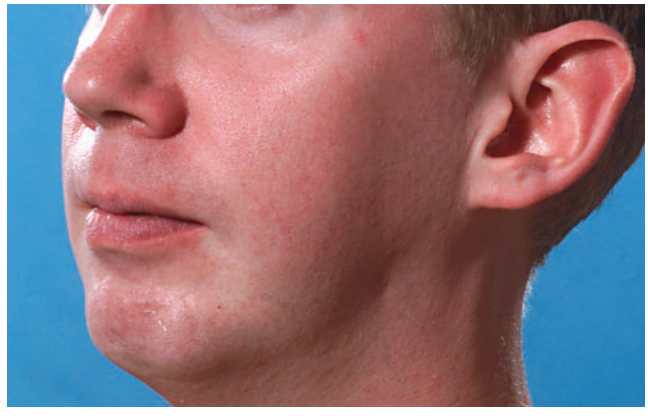


(i)

Figure 4 (h,i) subsequent relapse

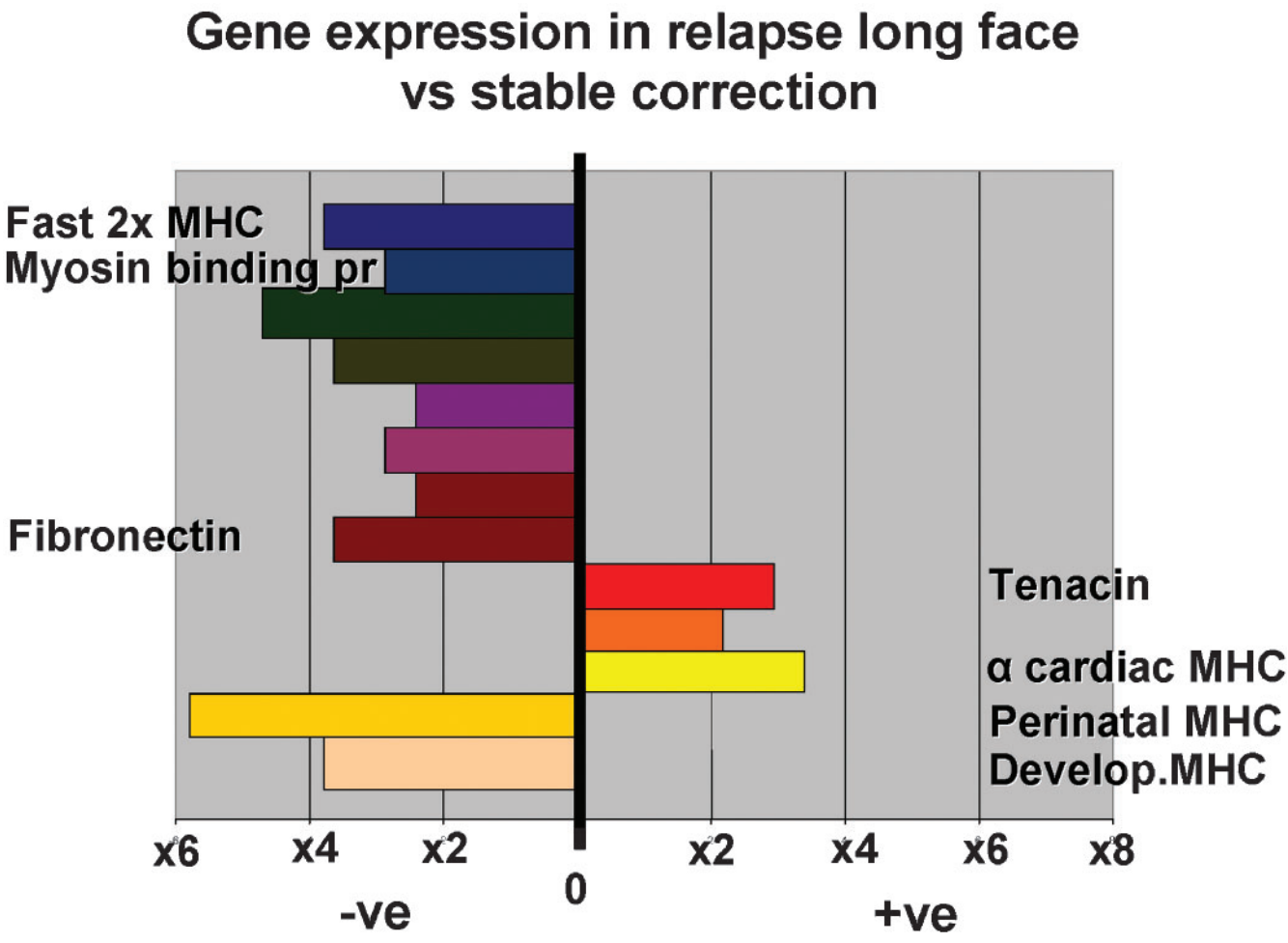


Figure 5 A selected printout of the relative gene expression of a bimaxillary correction which relapsed (using a stable correction as the comparator)

change to a more efficient musculo-occlusal relationship. However, based upon the very limited results of gene expression analysis it is noteworthy that those patients who have experienced considerable relapse have evidence of a pre-operative deficiency of the developmental isoforms in comparison to those patients in whom a stable result was achieved (Figure 5). Again, it must be emphasized that further work on a larger sample is indicated, but there remains the possibility of the development of a prognostic index, particularly for those high angle, Class II cases in whom there is a significant bowing of the lower border of the mandible and the presence of a large antegonial notch in whom the relapse potential is known to be increased.

The future

As our knowledge and understanding of how muscle adapts in response to treatment increases, so too does the possibility of experimental manipulation of muscle development.

The ability to grow muscle from human explants in the laboratory is now extremely successful and the gradual transition from individual muscle myoblasts through to fusion and maturation into multinucleated myotubes can be readily demonstrated (Figure 6). The possibility of manipulating human cells, for example, in conditions such as muscular dystrophy in which there is an inherent lack of the protein dystrophin remains a possibility. Reimplantation of such adaptive cells would be readily acceptable by the host as the original harvested cells were from the patient's own tissues. Early animal experiments have shown that reimplantation of myoblasts on soluble glass fibre scaffolds when placed in the hind limb of the MDX (muscular dystrophy) mouse, has shown that these cells continue to develop and produce normal dystrophin.

The ability to replace lost muscle tissue through tissue engineering has many potential uses, for example, in the

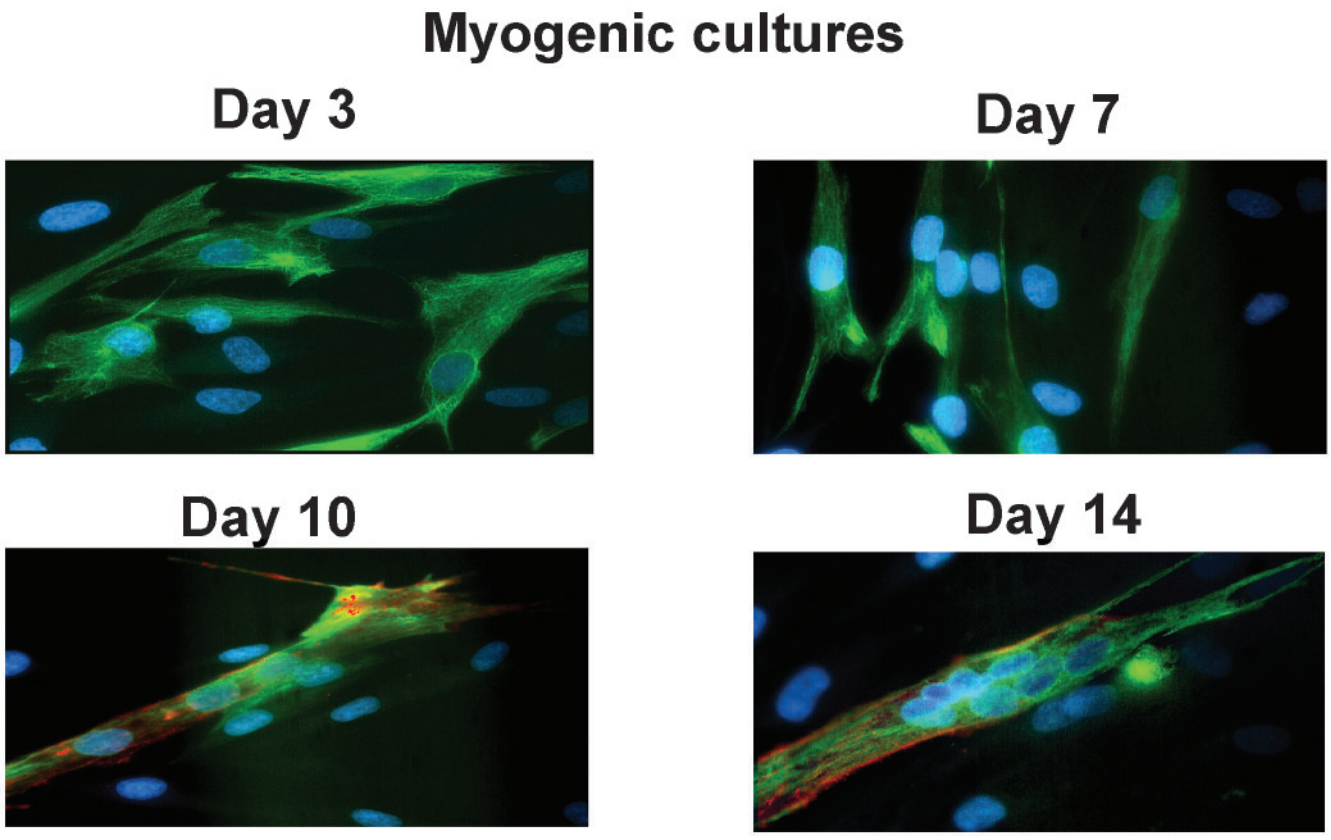


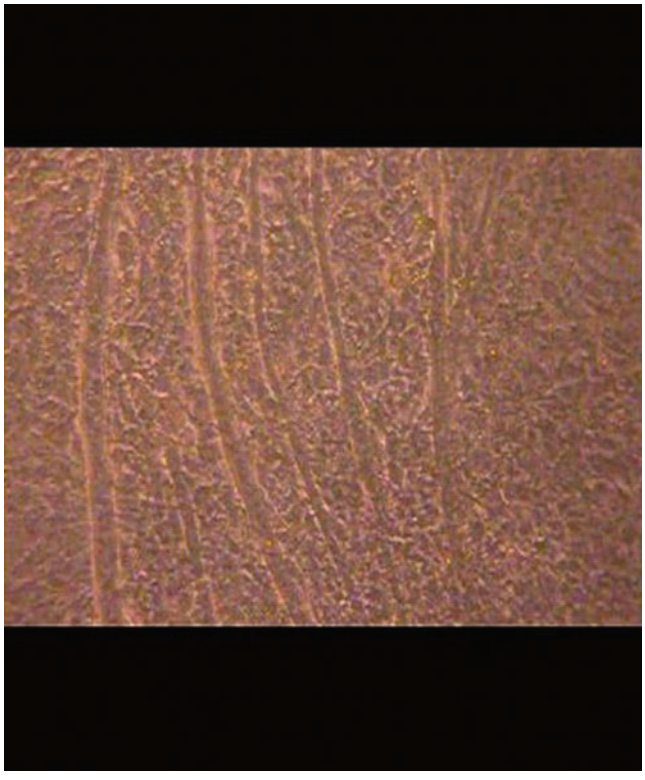
Figure 6 Human myoblasts evident in culture at day 3, which divide and align by day 7, and fuse to form primitive multinucleate myotubes by day 10. Development of contractile capability evident by presence of red stained sarcomeric actin at day 14

management of congenital deficiencies such as hemifacial microsomia or following traumatic or pathological tissue loss as a consequence of oncology. At present such replacement of muscle tissue involves free tissue transfer with all the inherent problems associated with the morbidity of the donor site. Growth of sheets of muscle cells on fibrin/thrombrin gels has shown that they continue to mature into primitive multinucleate myotubes, which subsequently can 'roll up' to resemble immature muscle fibrils (Figure 7). It has also been demonstrated that it is possible to stimulate these so-called 'myooids' so that they demonstrate contraction. There remains, therefore, the problem of overcoming the mechanism by which regenerated muscle cells can be reimplanted back into an individual. Ideally, one needs a scaffold that can support growth and differentiation of myoblasts, but which subsequently dissolves without inducing any tissue inflammatory response and which possesses a dissolution rate which matches the growth expansion potential of the particular muscle tissue. Previous work in our laboratory has demonstrated that phosphate based glass fibre rods provide a mechanism for the construction of such scaffolds[11] (Figure 8) and thus has enormous potential for the future.

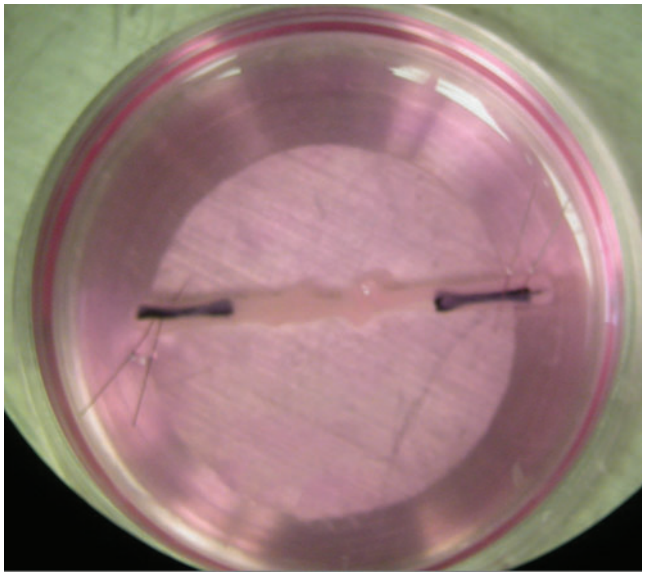
Conclusions

This article has attempted to provide contemporary thinking, and evidence with regard to the role of muscles in the aetiology and development of facial deformity, particularly in the vertical dimension, the response to treatment and ways of enhancing the tissue response. In summary:

- The question as to whether muscles play a part in the control of facial form remains unanswered, although increasing evidence would indicate that the muscle response is likely to be more adaptive to underlying skeletal development rather than a primary driving force.
- We possess an increased understanding of the mechanism of the adaptive response in muscle and this should lead to a greater understanding of our clinical management of cases in which treatment requires a direct adaptive response within the muscle tissue.



(a)



(b)

Figure 7 (a) Cultured masseter muscle growing on fibrin/thrombin gel with (b) subsequent contraction to form a rudimentary fibre (myooid)

- Finally, the prospect of developing new ways of engineering human skeletal muscle tissue remains an exciting possibility for the future.

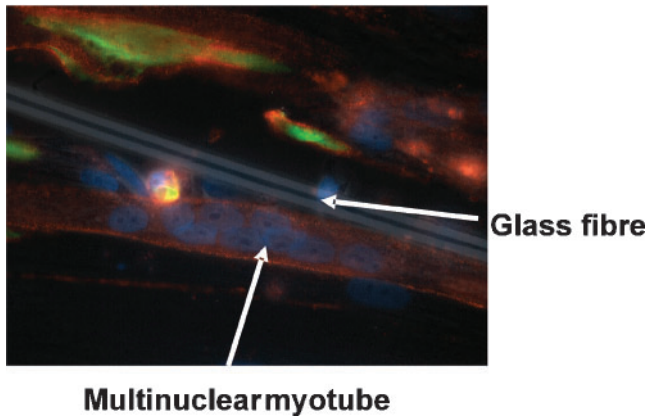


Figure 8 A primitive muscle fibre growing on a phosphate-based glass fibre scaffold

Acknowledgement

All the studies based at the Eastman Dental Institute and quoted in this essay were undertaken with the approval of the appropriate research and ethics committee.

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