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The need to more precisely define aspects of skeletal muscle regeneration☆

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ABSTRACT

A more precise definition of the term 'skeletal muscle regeneration' is required to reduce confusion and misconceptions. In this paper the term is used only for events that follow myofibre necrosis, to result in myogenesis and new muscle formation: other key events include early inflammation and revascularisation, and later fibrosis and re-innervation. The term 'muscle regeneration' is sometimes used casually for situations that do not involve myonecrosis; such as restoration of muscle mass by hypertrophy after atrophy, and other forms of damage to muscle tissue components. These situations are excluded from the definition in this paper which is focussed on mammalian muscles with the long-term aim of clinical translation to enhance new muscle formation after acute or chronic injury or during surgery to replace whole muscles. The paper briefly outlines the cellular events involved in myogenesis during development and post-natal muscle growth, discusses the role of satellite cells in mature normal muscles, and the likely incidence of myofibre necrosis/regeneration in healthy ageing mammals (even when subjected to exercise). The importance of the various components of regeneration is outlined to emphasise that problems in each of these aspects can influence overall new muscle formation; thus care is needed for correct interpretation of altered kinetics. Various markers used to identify regenerating myofibres are critically discussed and, since these can all occur in other conditions, caution is required for accurate interpretation of these cellular events. Finally, clinical situations are outlined where there is a need to enhance skeletal muscle regeneration: these include acute and chronic injuries or transplantation with bioengineering to form new muscles, therapeutic approaches to muscular dystrophies, and comment on proposed stem cell therapies to reduce age-related loss of muscle mass and function. This article is part of a directed issue entitled: Regenerative Medicine: the challenge of translation.

1. Introduction

One aim of this paper is to discuss semantics with respect to the rather casual use of the word 'regeneration' of skeletal muscle in the literature, as applied to various forms of damage and changes in muscle mass in mammals. Wide use of 'regeneration' for a range of very different cellular processes can lead to confusion and wrong assumptions. Classic muscle tissue regeneration involves myofibre necrosis (Fig. 1A). This results in a sequence of cellular events including inflammation and myogenesis to form new muscle to replace the damaged portion of the original tissue. Epigenetic regeneration is another process that occurs after limb amputation in some species (Godwin et al., 2013) and also in mammals during deer antler regeneration (Li et al., 2014). While epimorphic regeneration of skeletal muscle (that involves formation of a blastema) occurs in vertebrates such as amphibians and other species, it is not normally a feature of mature mammals (Brockes and Kumar, 2008) and thus will not be considered further here. A more detailed account of the cellular events involved in tissue and limb (epimorphic) regeneration and new muscle formation is provided elsewhere (Grounds, 2011).

In this paper, the term 'regeneration' is applied only to situations where myofibre necrosis has first occurred. Unfortunately, the same term 'muscle regeneration' is also used to describe 'repair' after damage to other components of muscle tissue, such as sarcomeric structure within myofibres (*e.g.* Z-band streaming), or interstitial extracellular matrix (ECM): this is very different to classic muscle regeneration.

Another situation where the term 'regeneration' is sometime misused relates to myofibres that undergo hypertrophy to return

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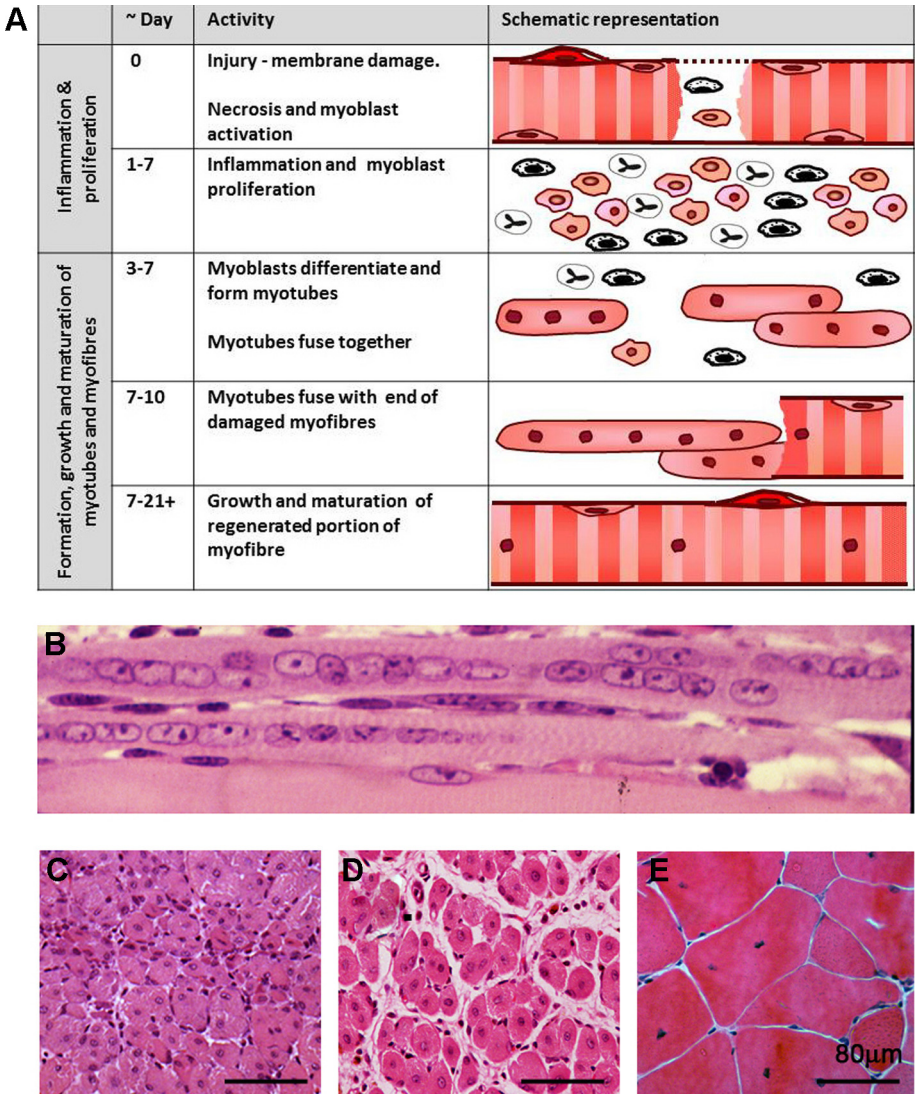

Fig. 1. The progression of cellular events in skeletal muscle during regeneration in response to myofibre necrosis, and examples of central myonuclei. (A) The time course of regenerative events after necrosis of a portion of a myofibre. Within 1 day of damage, necrosis is evident histologically as fragmented sarcoplasm and the presence of inflammatory cells. Coincidently there is myoblast activation and proliferation (by day one) and myoblast differentiation and fusion into myotubes occurs mainly between days 3 to 7. The myotubes fuse with more myoblasts and each other over the next few days, and by 10 days myotubes have fused with the ends of the damaged myofibres; inflammation decreases by this stage. Subsequently, myotubes and newly repaired segments of damaged myofibres undergo hypertrophy and mature to attain a stable adult size by about 3 weeks (adapted from Radley-Crabb et al., 2014). Regenerated myofibres in mice are identified by the presence of central myonuclei (*i.e.* myonuclei that are displaced and not in the classical sub-sarcolemmal peripheral position) that persist for many months. (B) Chains of central myonuclei are conspicuous in several newly formed myotubes/myofibres in a longitudinal section of adult mouse muscle regenerating after experimental injury: a portion of an undamaged myofibre with peripheral nuclei is shown at the bottom of the field [stained with Haematoxylin & Eosin (H&E)]. (C–E) Transverse sections through mature muscles stained with H&E showing myofbres with central or displaced myonuclei in three situations. (C) Dystrophic mdx muscle after endogenous myonecrosis showing recent regeneration with small newly-formed myotubes and larger older regenerated myofibres, all with central myonuclei. (D) Experimentally induced regeneration in young autograft of whole extensor digitorum longus muscle (from normal C57Bl/6J mouse aged 3 months) sampled at 10 days after transplantation, with many regenerated new myofibres with central myonuclei (from Shavlakadze et al., 2010b). (E) Myofibres of old quadriceps muscle (from sedentary male C57Bl/6J mouse aged 24 months), showing displaced myonuclei, even though there is no evidence of myonecrosis in these old muscles (Z. Soffe et al., manuscript accepted for publication).

to their original mass after some forms of muscle wasting (atrophy) that occurs in many clinical situations including disease, malnutrition, disuse, cachexia, denervation and ageing. Usually, the number of myonuclei remains unaltered throughout this process of decrease and increase in size, and only the myonuclear domain size changes: there is no involvement of satellite cells and this is essentially 'restoration' of muscle mass (discussed in Sections 3 and 4) Where pronounced hypertrophy occurs beyond normal size, numbers of myonuclei can increase at later stages due to fusion of satellite cells with the growing myofibre (in the absence of myonecrosis) to maintain the myonuclear domain (see Sections 3 and 4). Such exercise (or drug) induced hypertrophy is 'adaptation' and should also carefully be distinguished from the classical skeletal muscle regeneration that follows myonecrosis *in vivo*.

It is also pertinent to comment on myogenesis in tissue cultured cells (since these are so widely used experimentally) where myoblasts fuse to form young immature myotubes: this is not a situation of regeneration but represents only early events of myogenesis. These myotubes are not equivalent to mature innervated myofibres and this *in vitro* situation lacks the many key interacting events (e.g. inflammation, vascular interactions, blood-borne factors, ECM, innervation) involved in regenerating muscle and full function *in vivo*.

More precise use of 'muscle regeneration' is recommended to clarify discussions, especially in the context of potential clinical