Developing cultured meat scaffolds of extruded vegetable-based proteins

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ABSTRACT

Muscle cells from animals can be cultivated in cell culture medium, but to be used as a meat food product, they need a solid matrix to grow on that can also contribute to the texture. In this project we have created promising fibrous growth substrates from extruded plant based proteins that the cells are able to attach to and grow on. Cultured meat is still far from a commercial product, but may, in the long run, give even tastier, healthier and more environmentally friendly meat products.

INTRODUCTION

Global meat consumption per capita doubled between 1961 and 2011[1], and world meat production is expected to need to double by 2050[2]. Current meat production is one of the largest contributors of global green house gas emission and land use. Consequently, we are eating more meat than is sustainable for our environment and health, and face major challenges for meat production in the future. One is to be able to meet the increasing demand, in particular in rapidly developing countries. Another is to make meat production environmentally sustainable. Cultured meat made from skeletal muscle grown outside of the animal's body is speculated to be a future and environmentally sustainable alternative meat production method.[3,4] In this project, we investigate a new approach to produce cultured meat by growing skeletal muscle cells on fibrous substrates made from vegetable-based protein.

MATERIALS AND EXPERIMENTAL PROCEDURES

Hemp, maize and pea protein fibers were extruded using a Goettfert Rheograph 2000 capillary rheometer. Water was used as plasticizer, and following composition optimization trials, maize and pea were processed with a moisture content of 50% and hemp 40%. A circular die with an aspect ratio of 5/0.5 (0.5 mm die diameter, 5 mm die length) was used. The extrusion apparent shear rate was maintained at 9200 1/s and the temperature at 80C on all heating elements. The extruded fibers were sterilized either in ethanol or with UV-ozone and incubated with C2C12 skeletal muscle cells for one week in DMEM cell culture media supplemented with glucose, 1% Pen Strep and 10% FBS. Cells were fixed in formaldehyde and stained to visualize actin cytoskeletal filaments (rhodamin phalloidin), and imaged by confocal microscopy.

RESULTS AND DISCUSSION

The extruded pea, maize and hemp fibers are shown in Figure 1. The extrusion of pea and maize featured a continuous output whereas processing discontinuities were recorded for hemp.

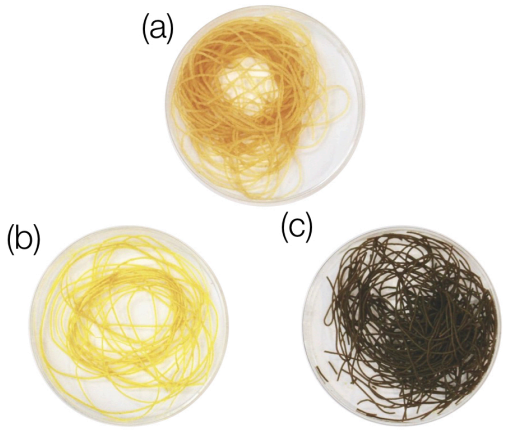

Figure 1. The extruded (a) pea, (b) maize and (c) hemp fibres.

Hemp protein fibers dissolved slightly in cell culture medium and were infected with bacteria. In addition, no muscle cells grew in hemp cultures even after sterilization. We suspect there is some impurity in the original hemp protein source that is released from the extruded fibers and is toxic to the cells. Fibers of maize and pea remained intact after the incubation period and the fibers sterilized in ethanol supported muscle cell attachment and growth, however bacteria were observed in the pea fiber samples. The only protein fiber showing no bacterial growth after one week incubation was the maize protein sterilized by ethanol. All the samples sterilized with UV-ozone contained bacteria, indicating that the intensity or the technique itself is not a suitable sterilization approach for these protein fibers.

It was observed that, after one week in cell culture, the muscle cells attached and proliferated best on maize protein fibers sterilized in ethanol (Figure 2), and began to align and fuse in preparation for myotube formation (Figure 3). Figures 2 and 3 show C2C12 skeletal muscle cells (in red) growing on top of, and beside, a maize protein fiber. Auto- fluorescence from the fiber is shown in green.

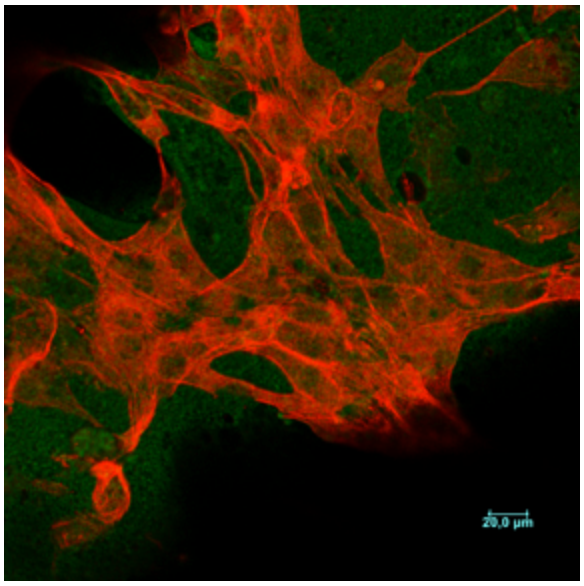

Figure 2. Skeletal muscle cells (C2C12) from rat growing on maize protein extruded fibers. Red=rhodamine phalloidin stained actin filaments. Green=auto-fluorescence from protein fibers.

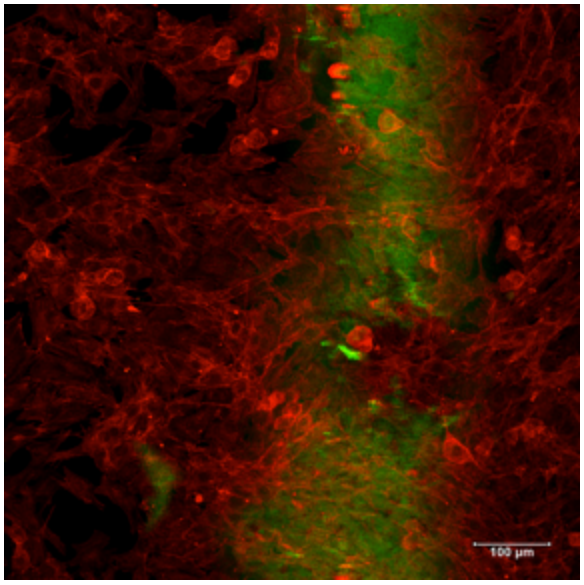

Figure 3. Skeletal muscle cells (C2C12) from rat growing on, but also beside, maize protein extruded fibers. Local regions of cell aligment and fusion to form myotubes are visible. Red=rhodamine phalloidin stained actin filaments. Green=auto-fluorescence from protein fibers.

Maize and pea protein are promising substrates for skeletal muscle cell growth, although a better sterilization protocol needs to been developed. In this respect, it would be beneficial to produce the fibrous substrates directly in a sterile environment. Longer culture times should be investigated to see if mature muscle fiber formation is possible to achieve in these constructs.

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