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Lab resource

Bovine annulus fibrosus cell lines isolated from intervertebral discs

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ABSTRACT

The adult bovine (*Bos taurus*) intervertebral disc is primarily comprised of two major tissue types: The outer annulus fibrosus (AF) and the central nucleus pulposus (NP). We isolated several primary cell lineages of passage (P) 0 cells from the AF tissue omitting typically used enzymatic tissue digestion protocols. The cells grow past p10 without signs of senescence in DMEM + 10% FCS on 0.1% gelatin coated/uncoated surfaces of standard cell culture plates and survive freeze-thawing. Preliminary analysis of the AF derived cells for expression of the two structural genes *Col1a1* and *Col2a1* was performed by PISH recapitulating the expression observed *in vivo*.

Resource table.

Name of resource	Adult bovine (*Bos taurus*) intervertebral disc annulus fibrosus (AF) cell lines.
Institution	Department of Biology, Clarkson University, 8 Clarkson Avenue, Potsdam, NY 13699, USA.
Person who created resource	Petra Kraus and Thomas Lufkin
Contact person and email	Petra Kraus, pkraus@clarkson.edu; Thomas Lufkin, tlufkin@clarkson.edu
Date archived/stock date	Established April 23rd 2016, frozen as passage 3 on May 9th 2016
Type of resource	Cell line derived from the annulus fibrosus tissue of the intervertebral disc of an adult *Bos taurus*
Link to directly related literature that employed/validated this resource	In press
Information in public databases	N/A, in press

1. Resource details

Several cell lines were isolated during independent experiments from dissected annulus fibrosus (AF) tissue of mature bovine intervertebral discs (IVD) *via* a reproducible non-enzyme driven protocol. The cell lines were frozen at low passage number and they recovered well after freeze-thawing (see Fig. 1). Preliminary characterization of the AF cells was carried out with bovine specific RNA probes derived from bovine genomic DNA using plate RNA *in situ* hybridisation (PISH) [1] for *Col1a1* and *Col2a1* expression, two structural proteins found in the mature IVD [2]. Less type-II collagen fibers were described for the outer AF in rabbit [3] correlating with a common notion that type II collagen is higher in the NP than the AF [2,4]. The dissected outer AF of mature bovine IVDs was the source for our AF cell lines and we did not detect *Col2a1* expression by either RNA *in situ* hybridization (SISH) [5–8] on sections of the outer AF tissue or by PISH on the cells derived from the outer AF, while *Col2a1* expression was very prominent in cells of the NP as shown by SISH on the same section (Fig. 2). The discrepancy between our findings and that of increased *Col2a1* expression in the bovine AF over the NP reported by Minoque et al. [9] might reflect differences in defining the AF. Minoque's Microarray expression analysis indicated increased expression of *Col1a1* in AF cells [9]. We see *Col1a1* expression in AF and NP cells by SISH *in vivo* and by PISH *in vitro* (Fig. 1).

2. Materials and methods

Skinned bovine tails were collected fresh from local abattoirs, remained chilled and were processed within 2 h. Tail pieces were immersed in 10% Povidone-Iodine solution, rinsed with tap water, followed by immersion in 70% EtOH prior to removing all fat and muscle tissue. IVDs were dissected away from adjacent vertebrae endplates, briefly dipped in 70% EtOH and rinsed with 1× PBS/10% Gentamicin prior to separating the outer AF from the remaining IVD tissue. Outer AF tissue was cut into smaller pieces using sterile procedures and placed in uncoated as well as 0.1% gelatin coated 35 mm culture dishes (Falcon). Sterile filtered FBS-HI with 10% Gentamicin and 5 μg/ml Amphotericin B (all GIBCO) was added prior to the incubation at 37C, 5% CO_2 and atmospheric O_2. After 24 h the FBS mix was diluted 1:1 with standard DMEM based growth medium containing 1× DMEM with 4.5 g/l glucose, 1× Pyruvate, 1× Glutamax, 1× nonessential amino acids, 10% v/v HI-FBS, 0.48% v/v Gentamicin (all GIBCO), 0.12 mM beta-mercapthoethanol (Sigma) and additional 5 μg/ml

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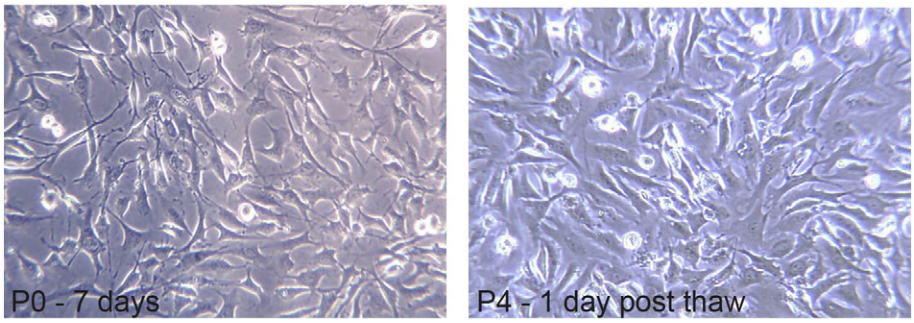

Fig. 1. Cells derived from the outer annulus fibrosus (AF) at P0 with 7 days in culture are shown on the left, while cells from the same cell line at P4 are shown on the right one day after a freeze thaw cycle. Images were taken using phase contrast at 200× on a Zeiss Primo Vert scope with a Moticam 2 2.0MP digital camera.

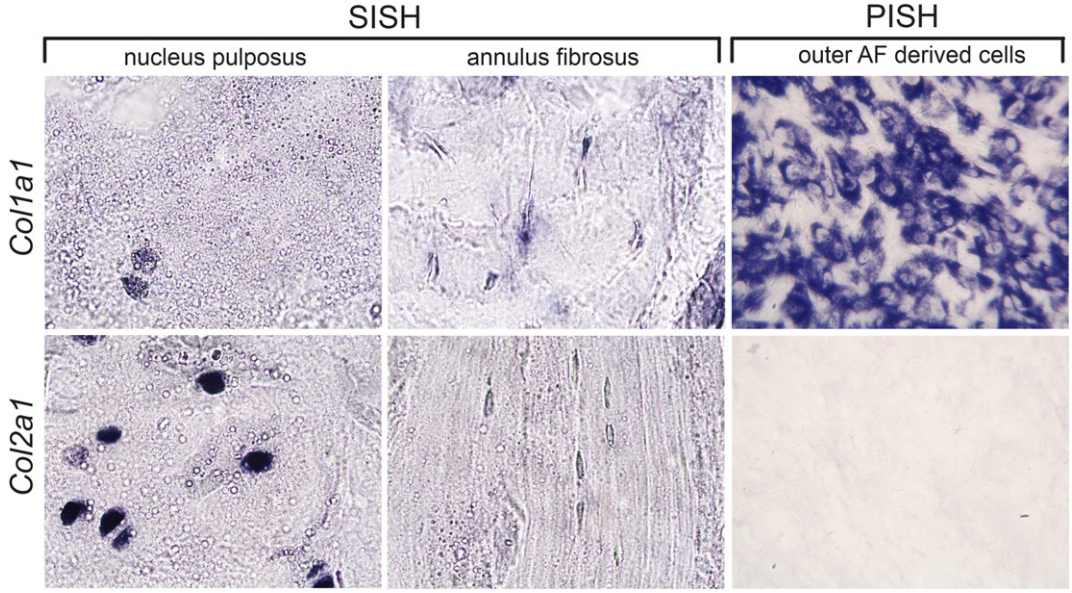

Fig. 2. 7um paraffin cross sections of the mature bovine IVD show cells expressing *Col1a1* mRNA by cells of the nucleus pulposus (NP) and outer annulus fibrosus (AF) by section *in situ* hybridization (SISH) and cells derived from this region of the outer AF by plate *in situ* hybridization (PISH). *Col2a1* expression was detected in the NP by SISH, but absent from AF tissue and cells derived therefrom. SISH images were taken at 400× on a MoticBA310 compound scope with a Moticam 1SP 1.3MP digital camera and PISH images at 200× on a Zeiss Primo Vert scope with a Moticam 2 2.0MP digital camera.

Amphotericin B and the tissue. Following 48 h of incubation cells had attached to the bottom of the wells and were expanded in fresh standard DMEM based growth medium (see above). Cell lines derived in such manner from AF tissue could be passaged with 0.05% Trypsin/EDTA (GIBCO) at 1:10 dilutions for more than 10 passages without slowing down in population growth or dramatic changes in morphology (Fig. 1). Early and late passages were subjected to plate RNA *in situ* hybridization (PISH) [1] for preliminary gene expression analysis (Fig. 2).

3. Verification and authentication

During embryogenesis, the AF part of the IVD is believed to be of sclerotomal origin [10,11]. Cultured cells derived from the outer AF of mature bovine caudal IVDs with our procedure were assayed for the expression of two major collagen genes *Col1a1* and *Col2a1*. The observed *in vitro* expression of these two genes mirrored the *in vivo* expression in cells of the mature the AF: Presence of *Col1a1* expression and absence of *Col2a1* expression in cells of the outer AF (Fig. 2).

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