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Research Article:

Bioimaging — a semi-quantitative tool to evaluate differentiation of human embryonic stem cells into chondrocytes

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Table S1. The schedule of supplementing chondrogenic medium with growth factors during the differentiation process (Yang et. al 2012).

DAY	WNT3a	Activin-A	FGF2	BMP4	Follistatin	GDF5
1	25	50				
2	25	25	20			
3	25	10	20	40		
4			20	40	100	
5			20	40	100	
6			20	40	100	
7			20	40	100	
8			20	40		
9						40
10						40
11						40
12						40
13						40

Table S2. Primary and secondary antibodies and their dilutions used for immunocytochemistry protocols.

Name	Company	Dilution
Primary antibody		
NANOG	Abcam	1:50
NANOG	Thermo Fisher	1:50
OCT-3/4	BD Pharmigen	1:50
SOX2	BD Pharmigen	1:50
SOX6	Abcam	1:50
SOX9	Abcam	1:50
Collagen type II	Abcam	1:100
B-catenin	BD Pharmigen	1:100
CXCR4	Abcam	1:100
BRACHYURY	Abcam	1:50
Chondroitin sulphate	Abcam	1:100
Heparan sulphate	Abcam	1:100
E-cadherin	Abcam	1:100
Secondary antibody		
Anti-rabbit IgG conjugated with AlexaFluor-488	Jackson ImmunoResearch	1:500
Anti-mouse IgG conjugated with AlexaFluor-488	Jackson ImmunoResearch	1:500

Anti-mouse IgM conjugated with AlexaFluor-488	Jackson ImmunoResearch	1:500

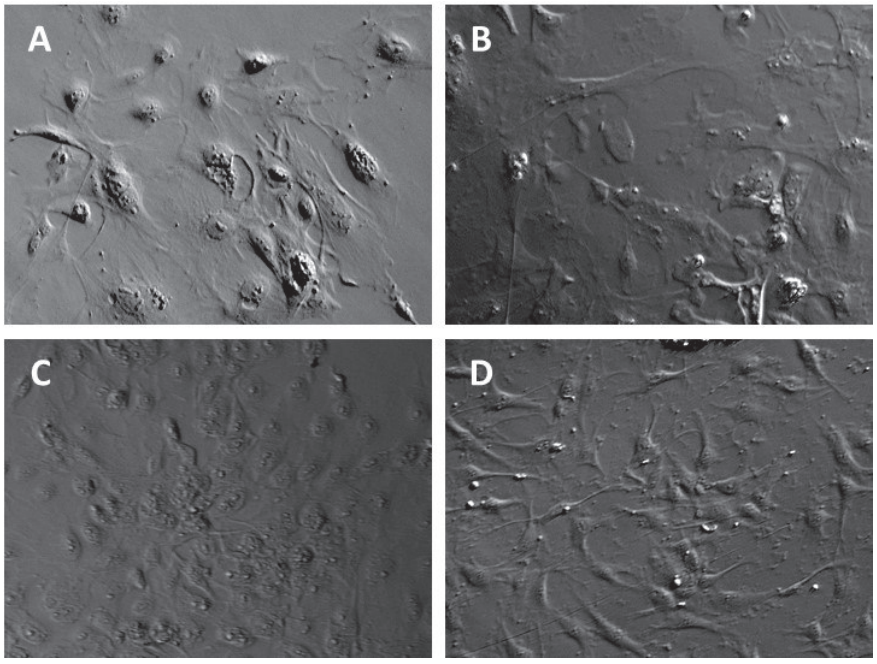

Figure S1. Differentiation protocols for obtaining chondrocytes from monolayer and EBs. Cells obtained from EBs supplemented with TGF-β_3 after 21 days (A) and exposure to various growth factors in monolayer cell culture after 14 days (B). As a control, human embryonic cells without MEFs were used (C) and chondrocytes obtained from waste material (D). Images were obtained using 100 × magnification.

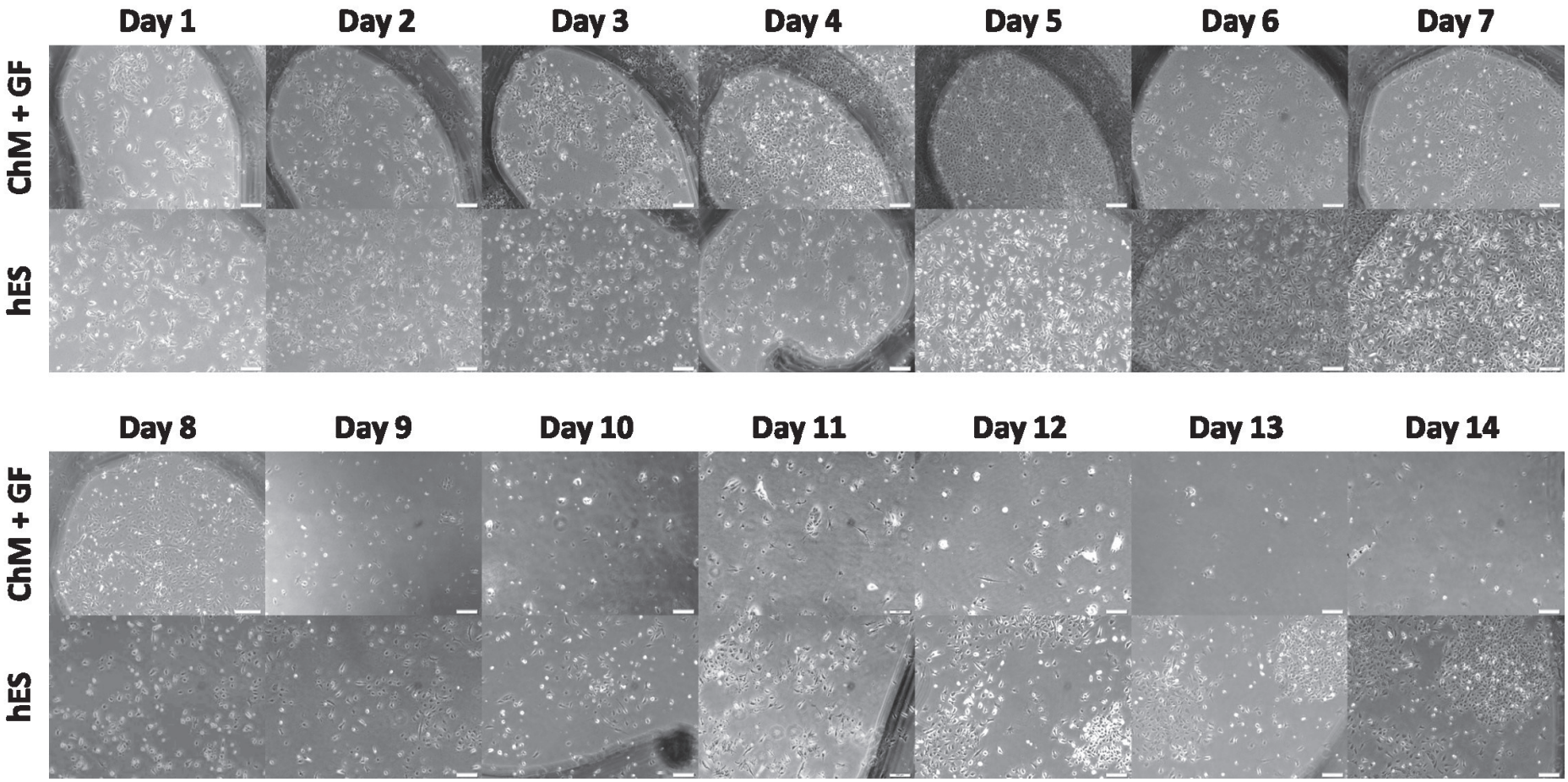

Figure S2. The observation of differentiation of hES cells into chondrocytes by monolayer protocol (14 days). During culture of ChM + GF cells, the growth of cells were increased what resulted in passage at 5^{th} and 8^{th}. On the following days it was observed decreasing proliferation of cells. What more, cells which did not undergo differentiation were detaching from surface of culture dish. As a control cell population hES were used. The white bar indicates 200µm. Pictures were taken under 100x magnification.