The study of differentiation of mouse embryonic stem cell into male germ cells using gelatin nanofibrous 3D scaffold and co-culture with Sertoli cells

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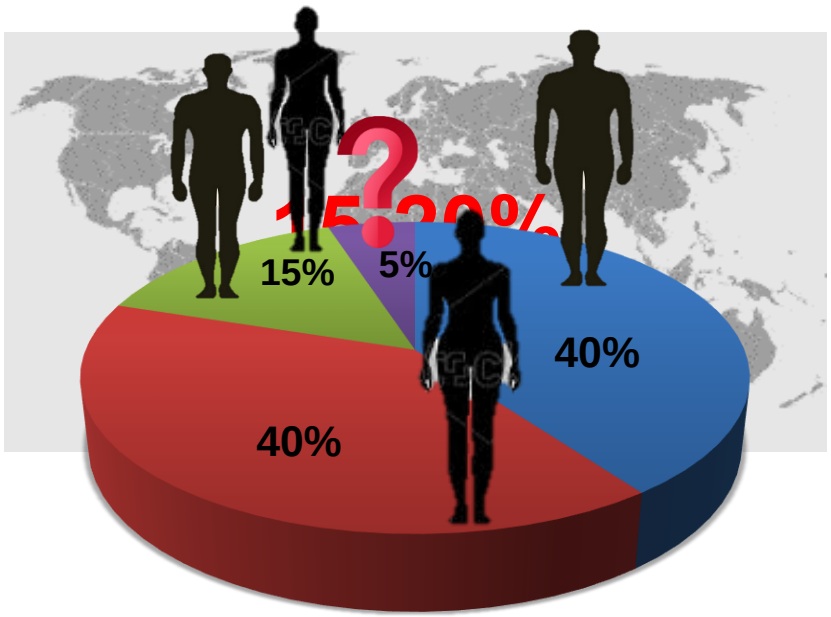
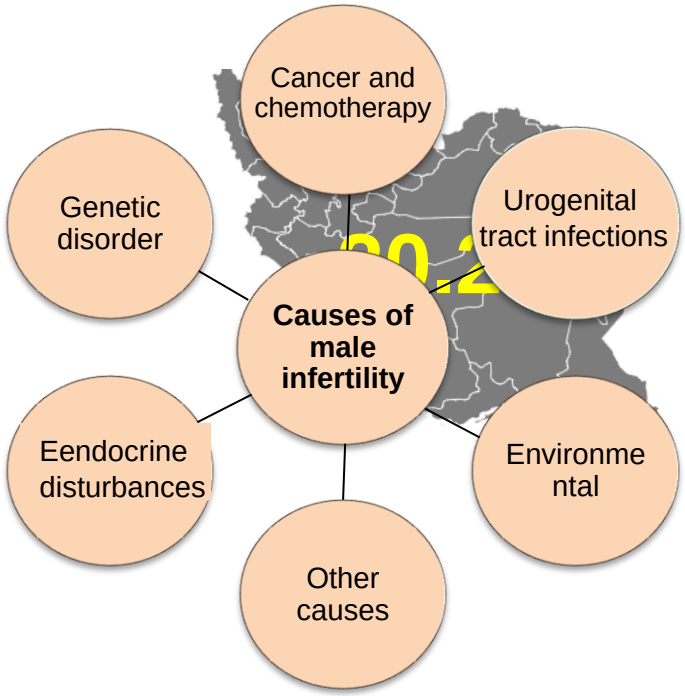
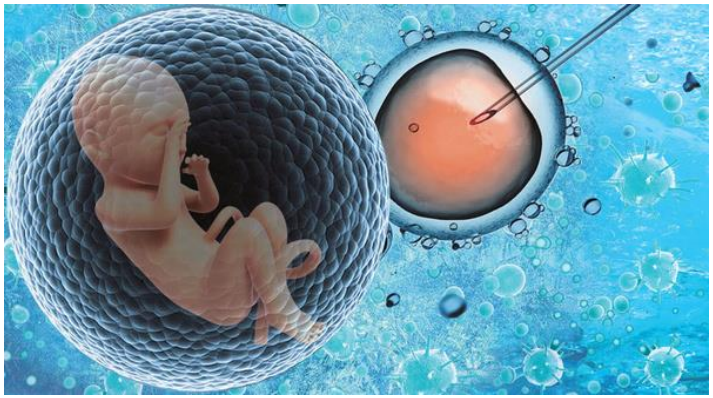
Male infertility



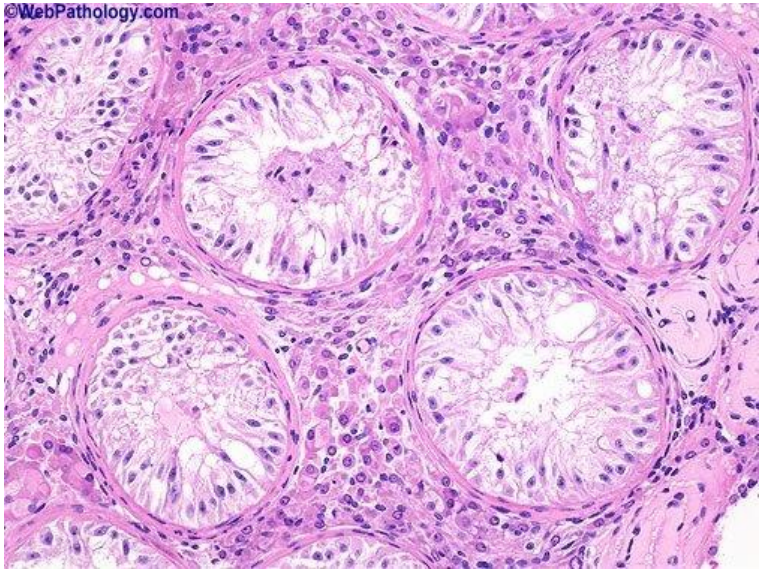




Esfandiari et al., Trend in Biotechnology, 2015
Singh et al.,Journal of Cellular and Molecular Medicine, 2011
O'Brien KL et al., Fertil Steril, 2010

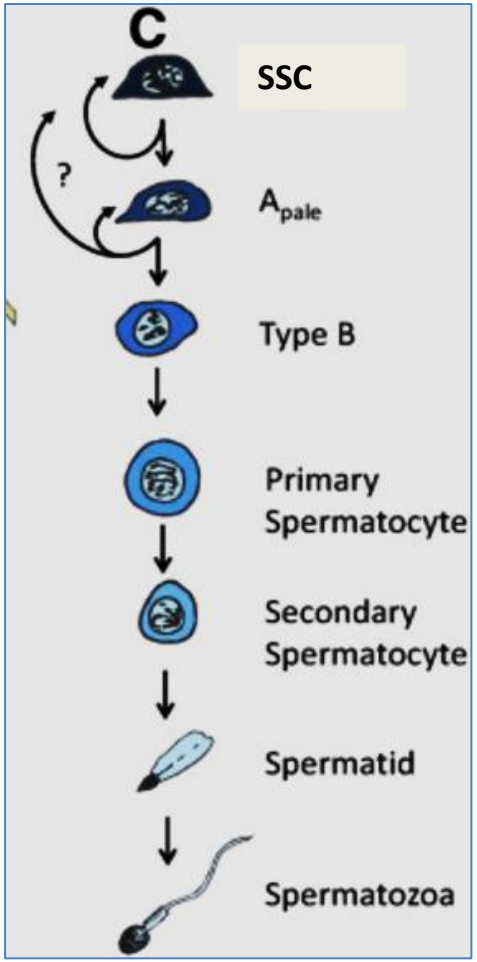
Male infertility

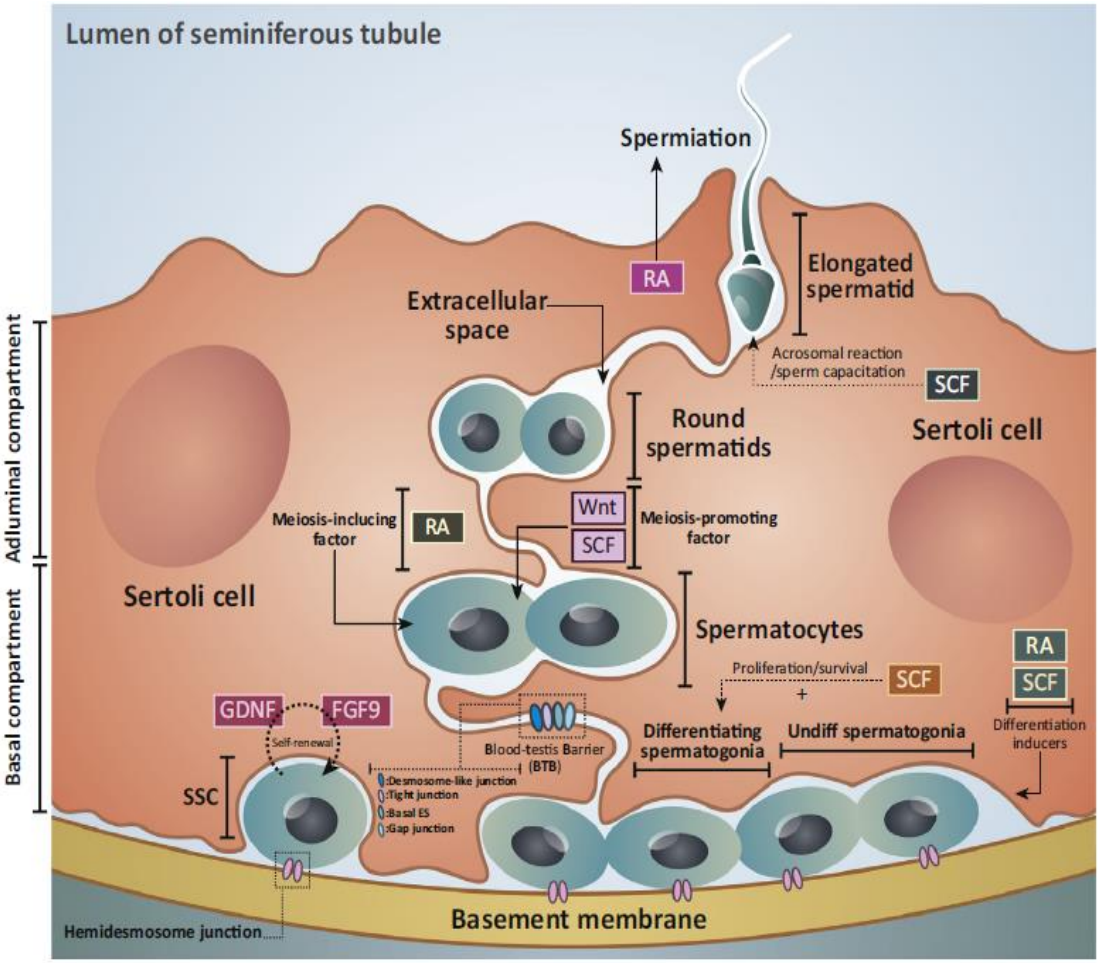
- **Non-obstructive Azoospermia**
  - **Sertoli-cell-only syndrome (SCOS)**
  - **Maturation arrest**
- **Klinefelter syndrome**
- **Gonadotoxic treatments**



Artificial Sperm Is Closer to Being a Reality

Spermatogenesis



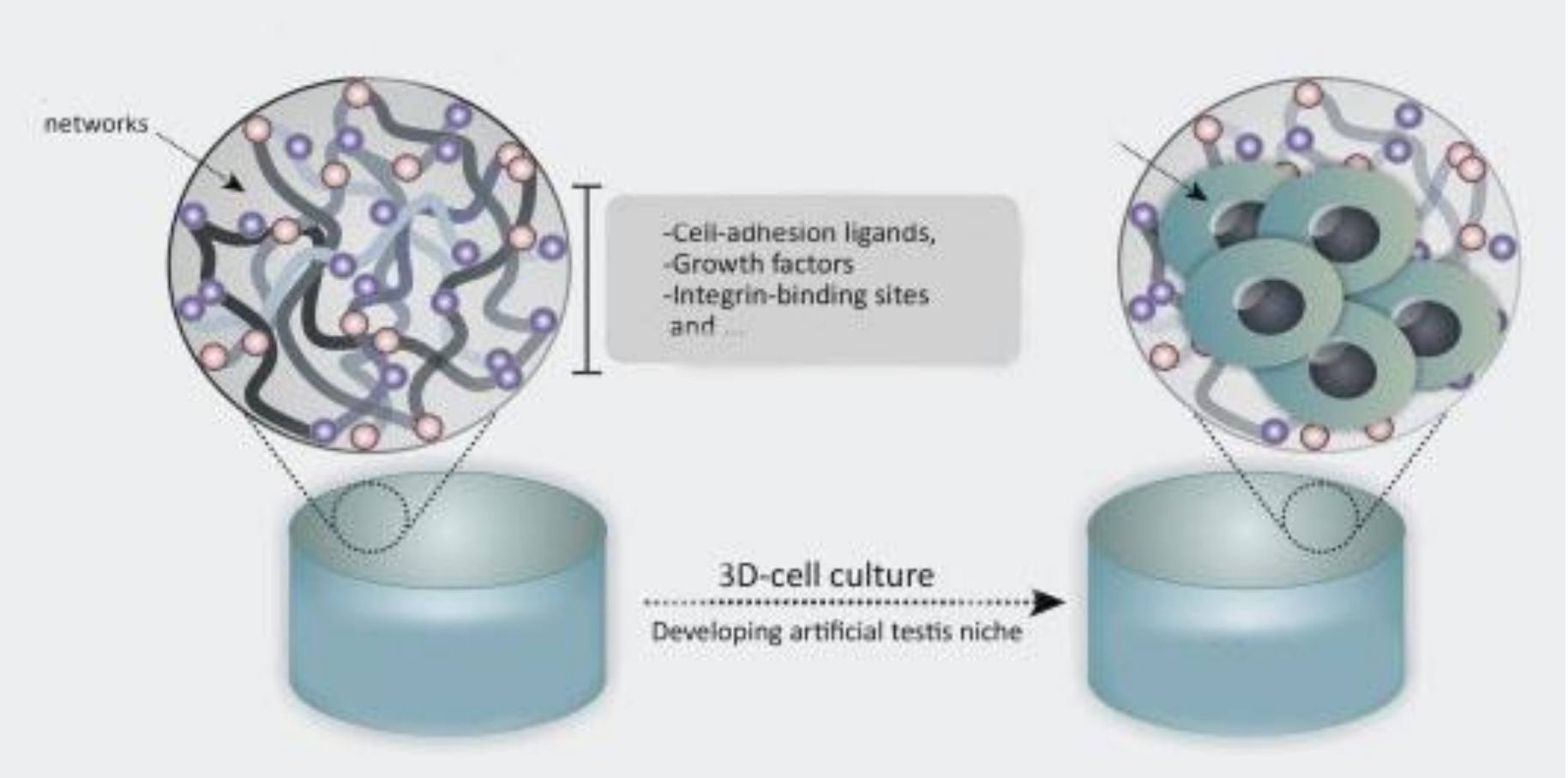


Esfandiari et al., Trend in Biotechnology, 2015
Oatley et al., Physiology Review, 2011
Kaavya et al., Int.J.Curr.Microbiol.App.Sci, 2017

In vitro culture systems for spermatogenesis

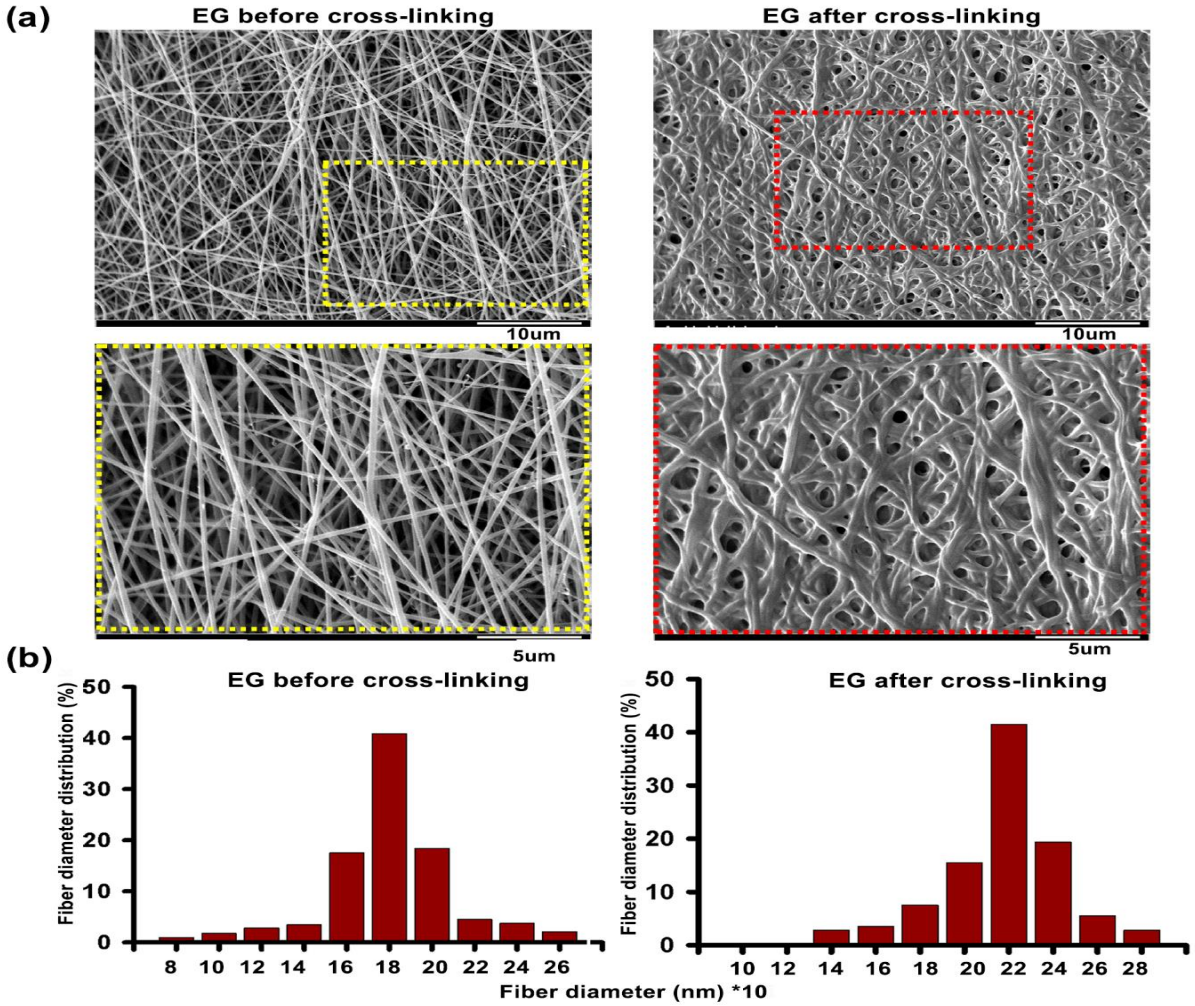
	Testicular 2D cell culture	Testicular 3D cell culture	Culture of testicular tissue fragments	
				
	Co-culture with Sertoli cells	Soft-Agar-Culture -System,	Hanging drop	Agar method (gas-liquid interphase)
Advantage	Feasible	Try to resemble the spatial environment	Maintain the spatial structure of the seminiferous tubule	Preserve Native niche Multicellular Cell-cell and cell-matrix interaction
Disadvantage	Do not maintain the spatial structure	Do not maintain the natural structure	Disruption of blood supply hampers nutrient and oxygen supplies	Disruption of blood supply hampers nutrient and oxygen supplies
	Iwanami et al., Theriogenology, 2006	Elhija et al., Asian J Androl, 2011	Jorgensen et al., Br J Cancer, 2014	Sato et al., Nature, 2011

Scaffolds



Iwanami et al.,
Theriogenology, 2006

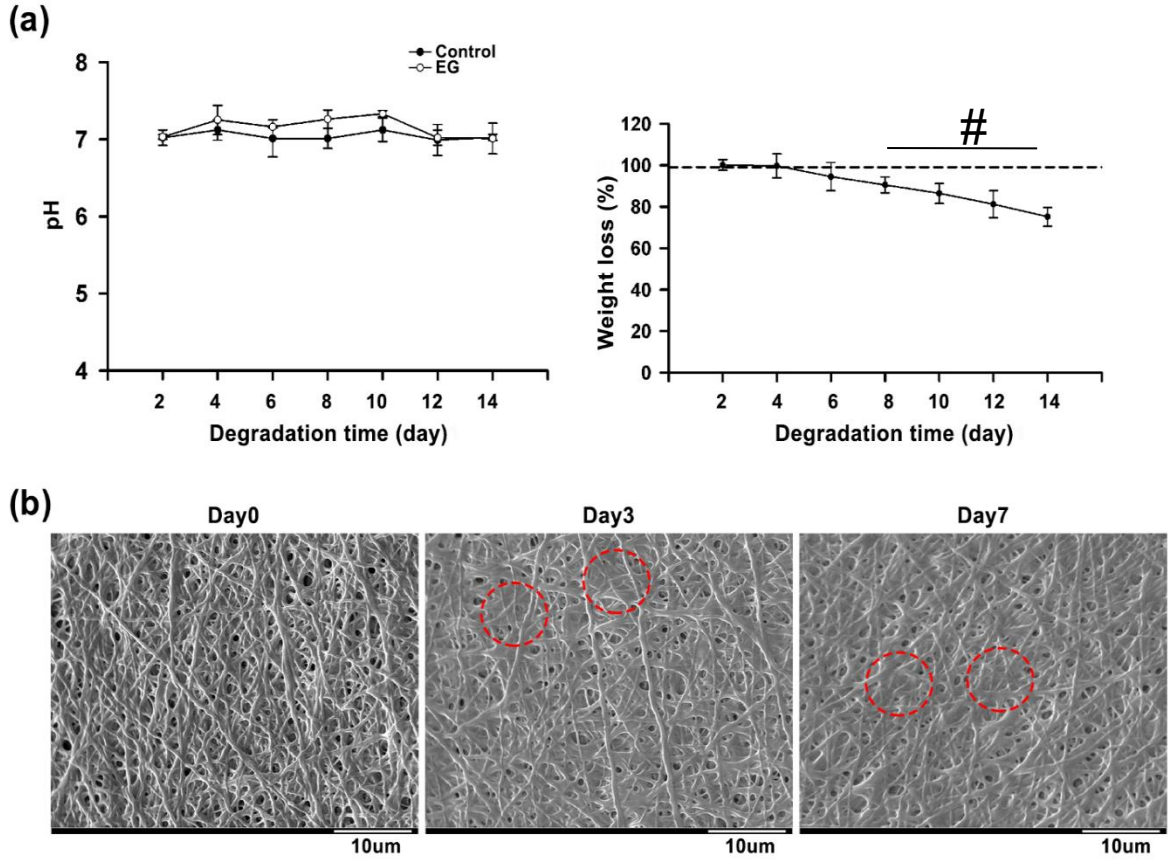
The morphology of the scaffolds



A, Morphology of electrospun gelatin scaffold before and after crosslinking with glutaraldehyde under the scanning electron microscope.

B, Distribution of fiber's diameter before and after crosslinking

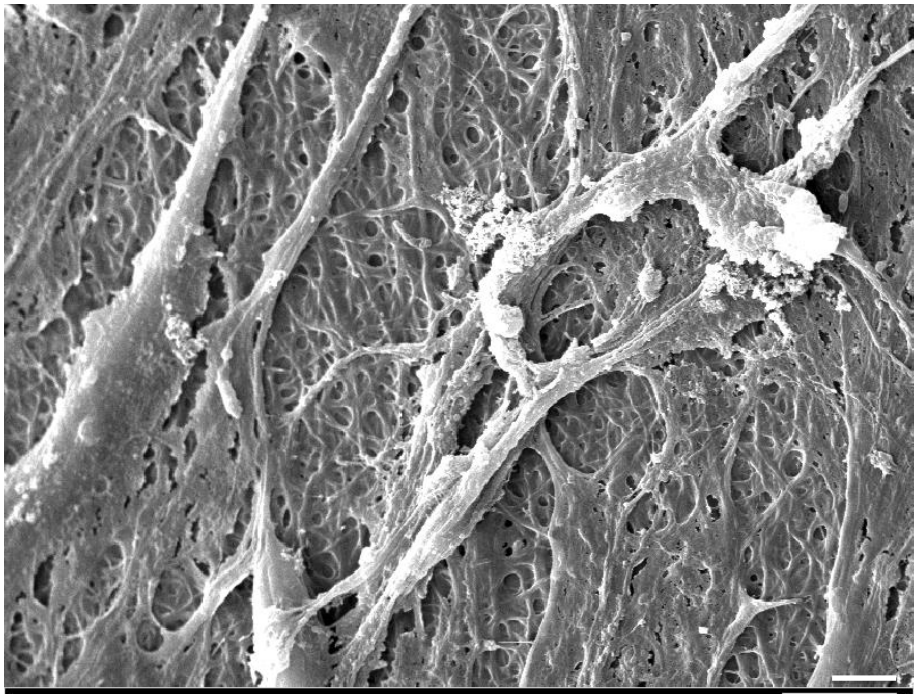
Biodegradability

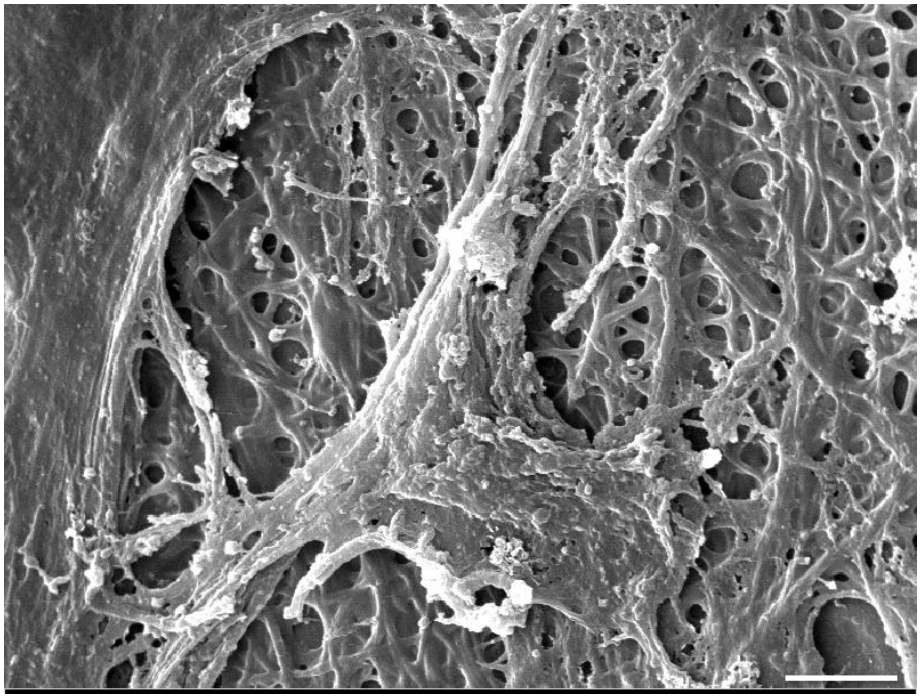


A, The pH value changes and weight loss (percentage) at days 2, 4, 6, 8, 10, 12, and 14 postdegradation time. Electrospun gelatin (EG) scaffold showed 24.8% ± 4.5% weight loss after 14 days with no changes in pH value compared with control (PBS).

B, SEM micrographs of EG scaffold at days 0, 7, and 14 postdegradation times. Red circles indicate the changes in the morphology of fibers. PBS, phosphate-buffered saline; SEM, scanning electron microscope

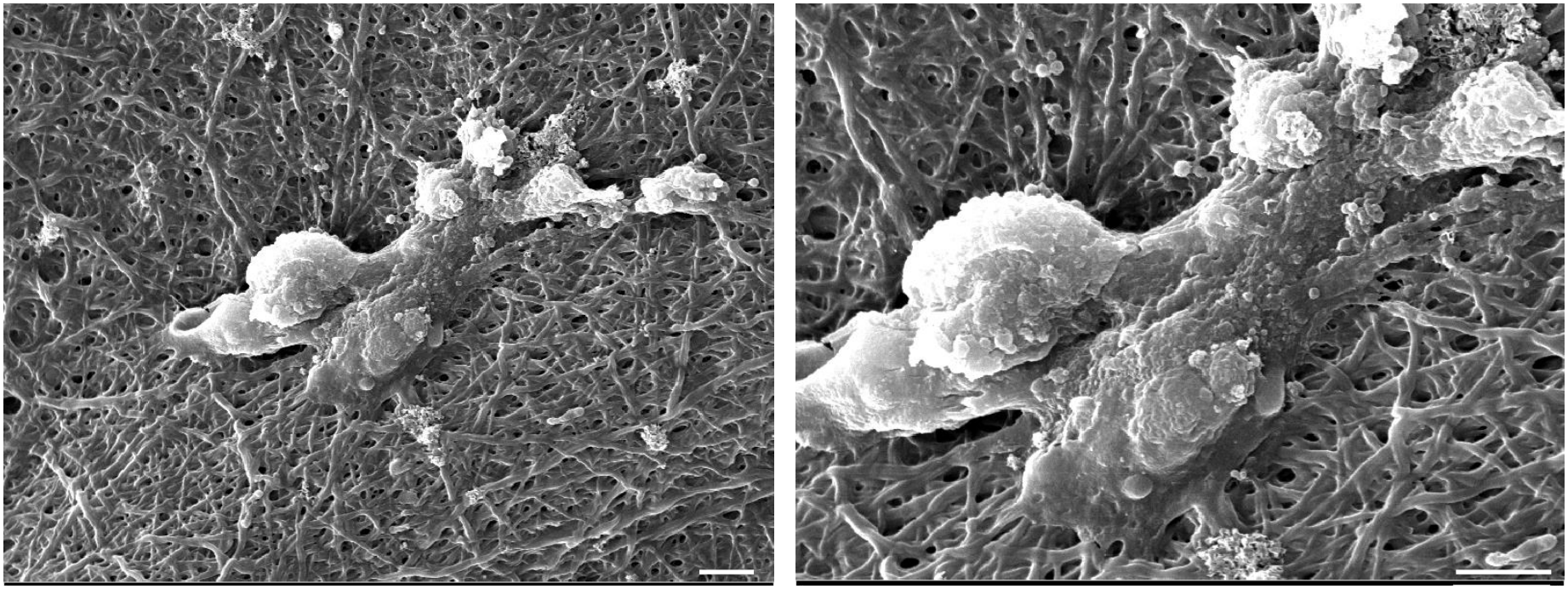
Cell adhesion properties of scaffold





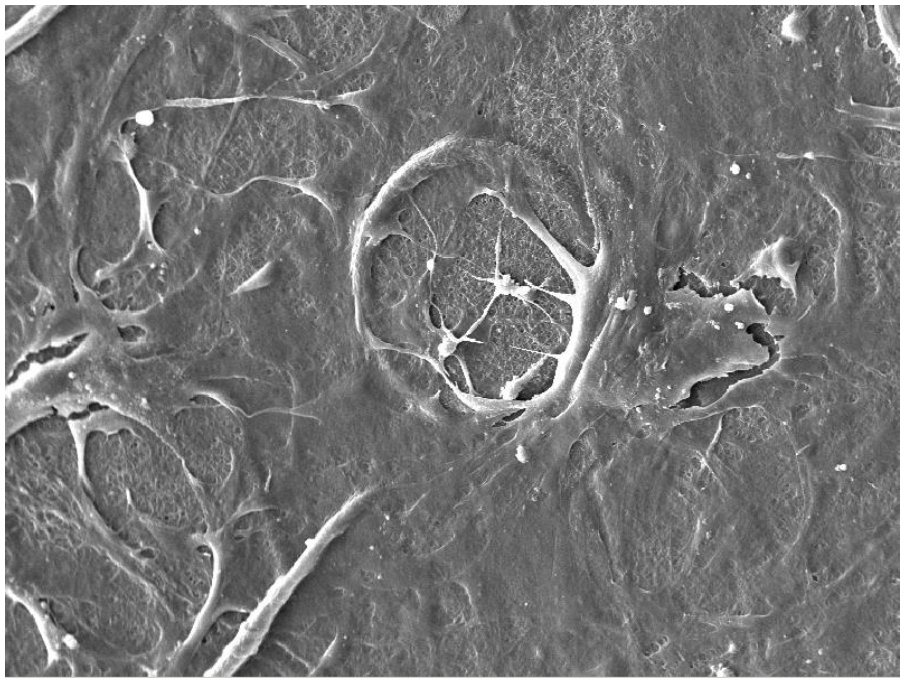
Scanning electron microscope (SEM) image showing the Sertoli cells seeded on the Gelatin nanofibrous scaffold after 1 week of cell culture. Scale bar represents 5 μm.

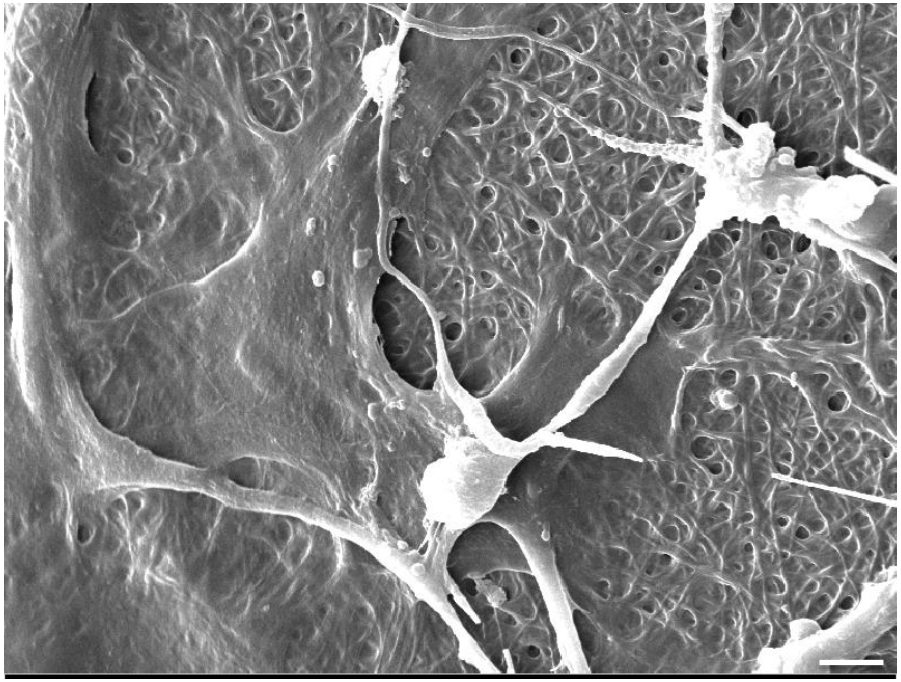
Cell adhesion properties of scaffold



Scanning electron microscope (SEM) image showing the embryonic stem cells (ESCs) seeded on the Gelatin nanofibrous scaffold after 1 week of cell culture. Scale bar represents 5 μm.

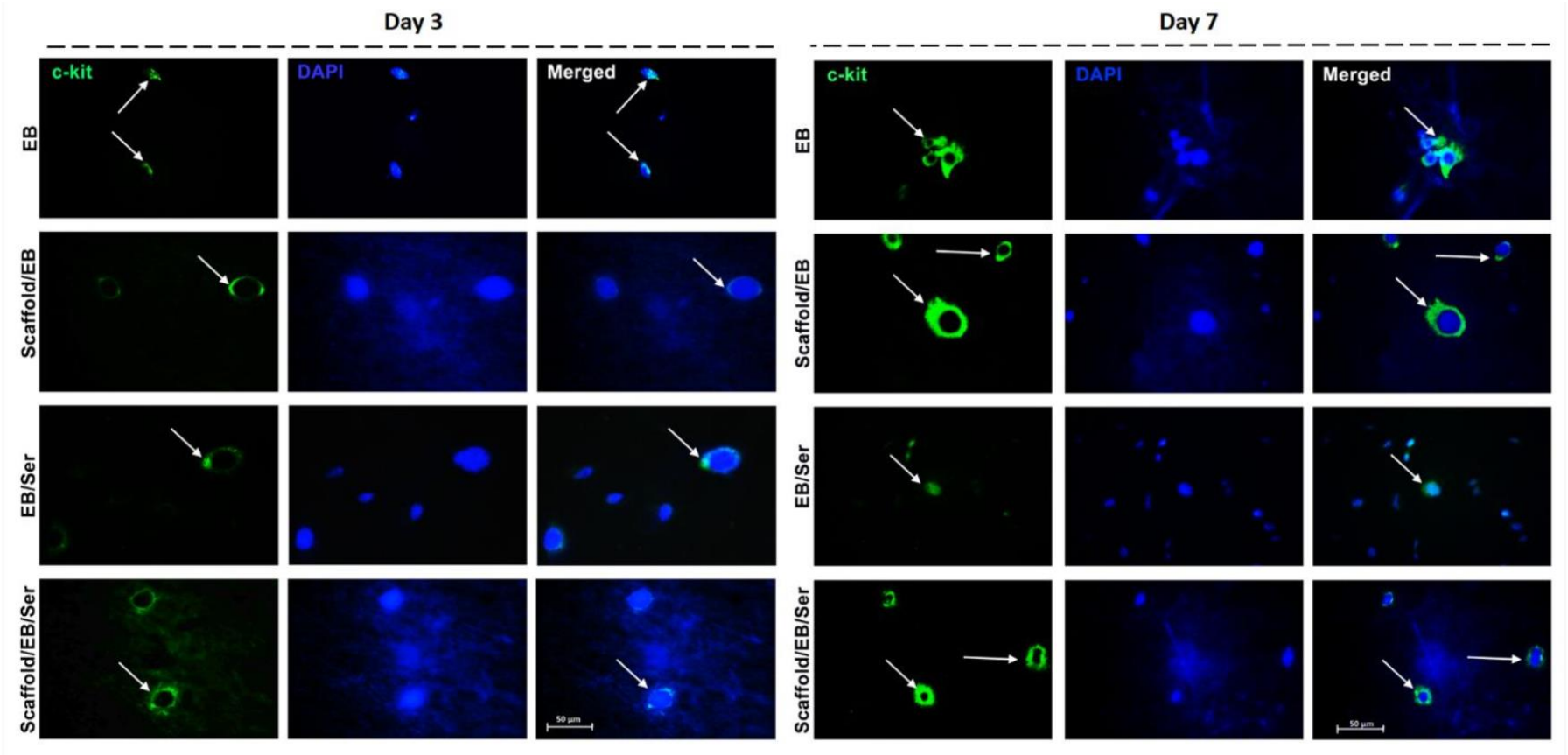
Cell adhesion properties of scaffold





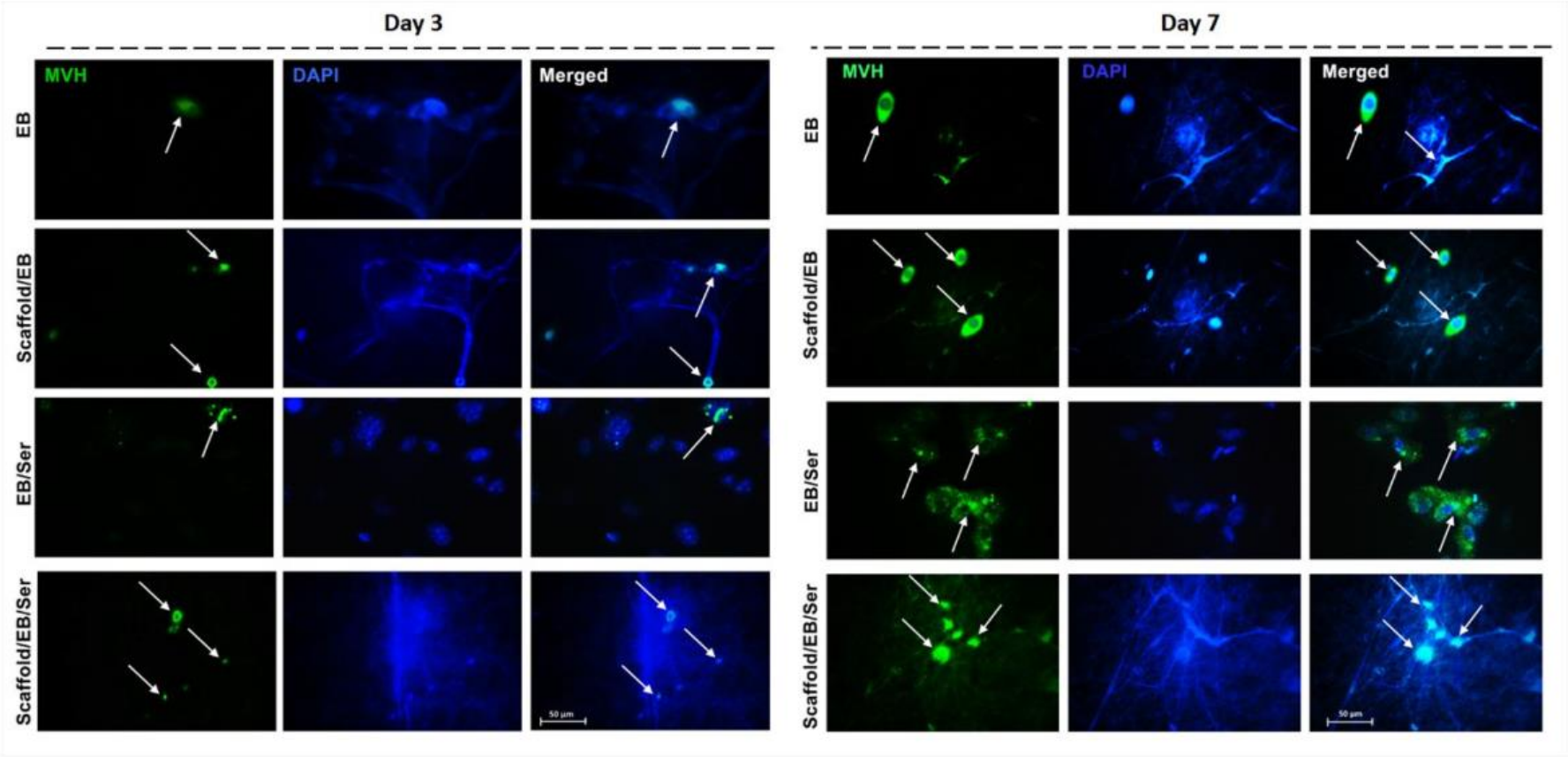
Scanning electron microscope (SEM) image showing the Sertoli cells and ESCs seeded on the Gelatin nanofibrous scaffold after 1 week of cell culture. Scale bar represents 5 μm.

stem cells differentiation toward germ cells and sperm producing cells



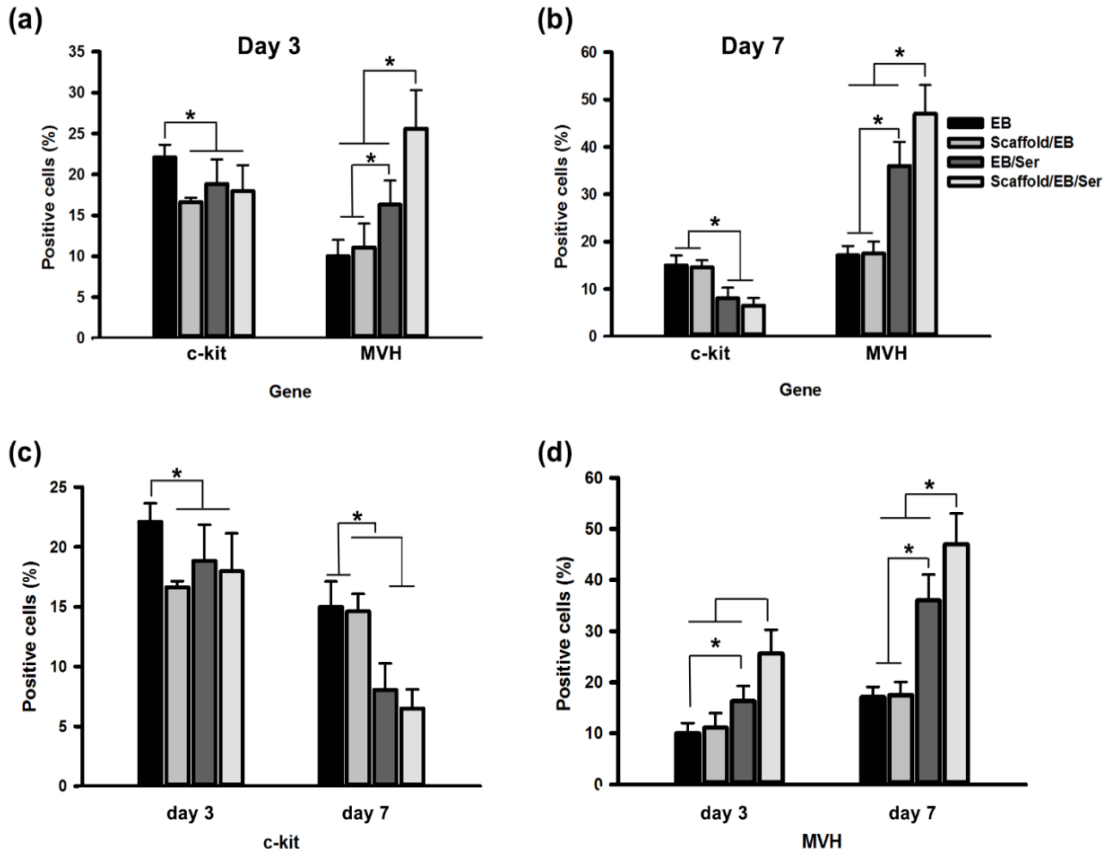
Immunostaining for c-KIT at day 3 (a) and 7 (b) of differentiation induction

stem cells differentiation toward germ cells and sperm producing cells



Immunostaining for MVH at day 3 (a) and (b) of differentiation induction

stem cells differentiation toward germ cells and sperm producing cells



Immunocytochemistry staining. Comparison of the percentage of cells expressing c-KIT and MVH at days 3 and 7 of differentiation

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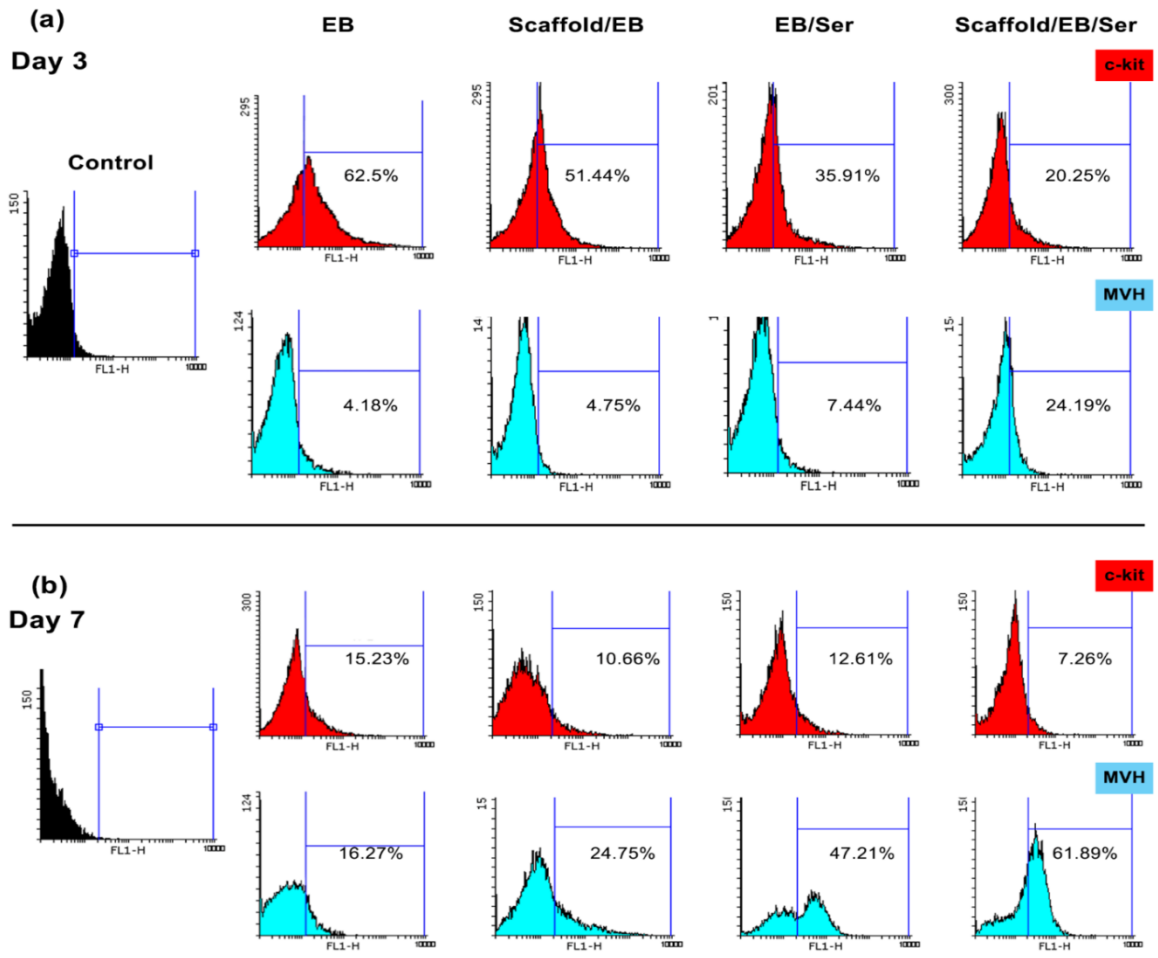
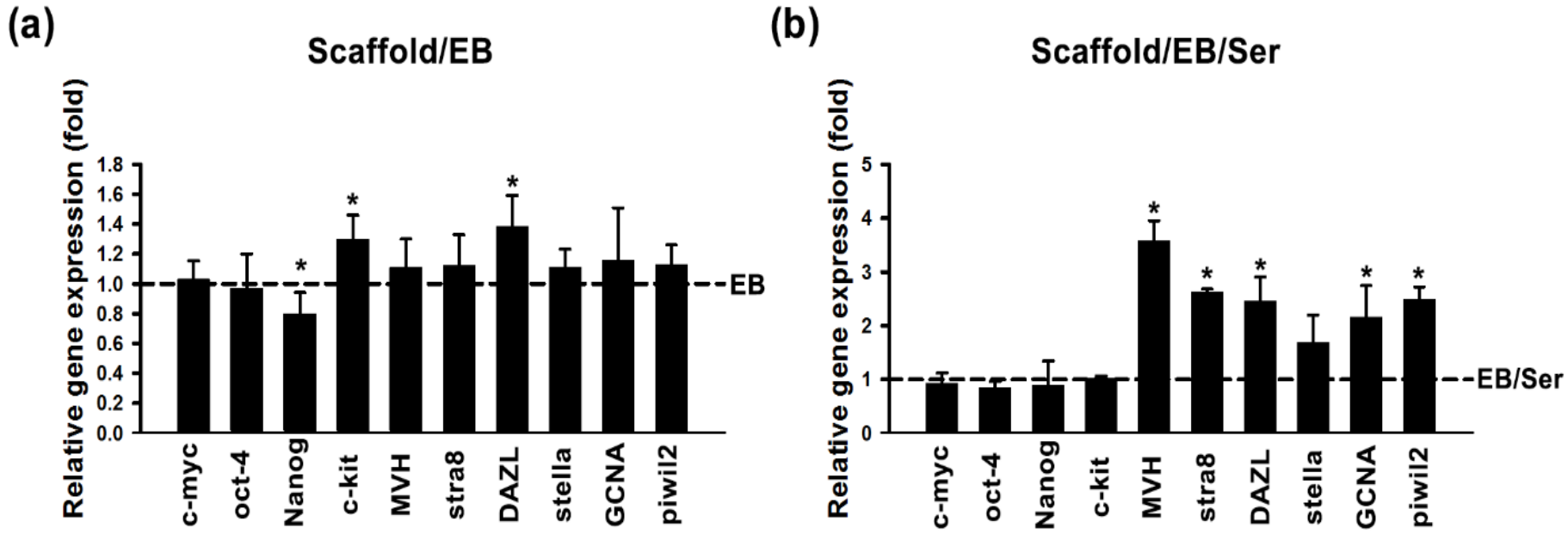


Flow cytometry analysis for phenotypic determination of the cells.

stem cells differentiation toward germ cells and sperm producing cells



Comparison of the relative expression (fold) of the pluripotency genes (Oct4, c-Kit, Nanog, c-Myc) and germline genes (GCNA, Stella, MVH, Stra8, Piwil2, DAZL) at day 7 of differentiation between the experimental groups.

Summary

- Our findings revealed that the EG scaffold can provide an excellent substrate bio-mimicking the micro/nanostructure of native seminiferous tubules, and a platform for Sertoli cells-Ebs communication required for growth and differentiation of ESCs into germline cells.