Austin Therapeutics

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Editorial

New Experimental Model for Basic Research in Stem Cell Field

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Received: October 29, 2014; **Accepted:** October 29, 2014; **Published:** October 30, 2014

Editorial

Mesenchymal stem cells (MSC) were isolated from a wide variety of tissues including bone marrow [1], umbilical cord blood [2,3], peripheral blood [4], adipose tissue [5,6], periosteum, trabecular bone, synovium, skeletal muscle, human fetal membranes [7], placental chorionic villi [8], amniotic fluid [9], fetal liver [10], deciduous teeth [11] and can also be found in menstrual blood [12].

The great interest in these findings is due to the promising possibility to use MSC in tissue engineering and gene therapy thanks to their plasticity and availability.

Recently a subpopulation of luteinized granulosa cells (GC) derived from infertile patients during their IVF procedures showed mesenchymal stem characteristics and multipotency [13]. This study used the addiction of leukemia-inhibiting factor (LIF) to the culture medium to promote the long-term survival of GC. LIF is a glycoprotein with different biological effects in various tissues and can be found both in fetal and adult human ovaries. Most previous studies on ovarian function resulted in a short-term cultures of GC or resorted to an immortalization using SV40 large T antigen [14,15], but changing the physiological aspects.

Granulosa cells were also cultured in 3D biological matrix made of collagen type I, which is a physiological component of ovarian tissue. These observations suggested the possible use of GC to create *in vitro* a surrogate follicle and induce the maturation of antral human oocytes [16].

In our recent study a heterogeneous cell population were isolated from human ovarian follicular fluid (FF cells) and cultivated in minimal medium conditions without any growth factors, including leukemia-inhibiting factor. FF cells showed a different morphology, such as fibroblastic, epithelial and also neuronal shape. In particular, the cells with characteristics similar to fibroblasts expressed many specific antigens of mesenchymal stem cells (i.e. CD90, CD44, CD105, CD73) and were negative for haematopoietic and epithelial markers (CD34, CD45, cytokeratins). We confirmed the multipotency of a subset of granulosa cells by *in vitro* differentiation studies (e.g. osteogenic, chondrogenic and adipogenic differentiation) [17].

Therefore we propose follicular fluid cells as a cheap biological experimental model for basic research in stem cells fields thanks to their clonogenic capacity, multipotency and availability without any growth factor addiction too.

One of biotechnological application of stem cells is in tissue engineering filed. Combining stem cells with biomaterial scaffolds provides a promising strategy for engineering tissues and cellular delivery to develop biological substitutes that can restore tissue functions. Even though there are some obstacles to the generation of functional tissues and this aspect limited their widespread clinical use, mesenchymal stem cells are the best lines available to a possible application in regenerative medicine. Thanks to their large capacity for self-renewal while maintaining their multipotency MSC can differentiate into a variety of cell types, including osteoblasts, chondrocytes and adipocytes.

In the field of reconstructive bone surgery many biomaterials were studied to heal critical-size bone defects due to trauma, tumor resection, and tissue degeneration.

We reported a promising affinity of FF cells derived from a biological fluid that is usually wasted during IVF procedures with different type of engineering scaffold. In particular we tested FF cells biocompatibility with gelatin cryogel, titanium scaffold and bioactive glass [18].

Gelatin cryogel is a promising biomaterial that favoured differentiation of bone marrow stromal cells. In fact, previously, it was showed a biomimetic strategy where differentiated human bone marrow stromal cells built their extracellular matrix onto gelatin [19,20]. Also FF cells cultured for 2 weeks onto gelatin cryogel showed cytoplasmic expression for vimentin (a cytoskeleton protein in mesenchymal cells) and membrane positivity for CD44 antigen (a marker of mesenchymal cells). These cells were enough able to grow till 60 μm of deepness in similar manner as bone marrow MSC [18].

Titanium is used for many years in reconstructive bone medicine and represents one of the major compounds used in bone implants [21]. Bioactive glass consists of a mixture of silicon dioxide, calcium oxide, and phosphate oxide. It is used as a bone substitute to fill defects and increase mechanical strength thanks to its surface reactivity, compatibility with surrounding bone tissue. It also acts as a suitable matrix for cell differentiation.

Cells were cultivated in proliferative culture conditions on three different biological supports: Titanium scaffold (Ti), Bioglass coated Ti scaffold (Bioglass-Ti) and control (TCP) for different times (1, 7 and 21 days respectively). In all conditions, it is possible to observe a

Citation: Omes C and Riva F. New Experimental Model for Basic Research in Stem Cell Field. Austin Therapeutics. 2014;1(2): 3.

trend of exponential growth in the first 7 days. At 21 days, the number of cells was less or equal to that found at 7 days ($p > 0.05$) because of cell confluence. The greatest number of viable cells on Ti and Bioglass-Ti than on TCP demonstrated that the biomaterials tested favored the growth of FF cells. Moreover it seems that the presence of bioglass promotes a better attachment and favors proliferation after 7 and 21 days compared to uncoated Ti scaffolds. FF cells were also cultured in differentiative conditions on Ti, Bioglass-Ti and TCP for 7 and 21 days, respectively and the activity of s (ALP) was detected. Again cells on Bioglass-Ti showed a greater ALP activity than on Ti and TCP indicating that Bioglass improves the cell differentiation process [22].

In the field of tissue engineering bioreactor systems provide both the technological means to reveal fundamental mechanisms of cell function in a 3D environment, and the potential to improve the quality of engineered tissues, by enabling reproducible and controlled changes of specific environmental factors.

For example Low-Intensity Ultrasound Stimulus (LIPUS) were used to accelerate the fracture healing in animal models [23,24] and in clinical studies [25,26].

We reported that FF cells could be a good model not only to study biocompatibility of engineering scaffold, but also to investigate the effect induced by mechanical conditioning. In fact in this study we observed that the application of LIPUS for 5 min per day was sufficient to induce a significantly enhanced cell proliferation [27].

Although FF cells showed typical mesenchymal aspects and were used for stem cell applications, they still maintained some granulosa cells properties. For this reason we are now studying the effects of hormonal stimulus on FF cells derived from infertile women to find possible correlation to the cause of infertility of patients from which FF cell were isolated during their IVF procedures. In particular we investigated the hormonal release (estradiol and progesterone) of FF cells *in vitro* after gonadotropin stimulation with drugs normally used for the controlled ovarian hyperstimulation in women undergone a cycle of IVF techniques.

Preliminary data suggested a different behaviour of FF cells *in vitro* dependent on the clinical history and the plasma levels detected in women at baseline. Moreover the progesterone release had a trend in culture related to the achievement of pregnancy *in vivo* during the IVF cycle from which FF cells were derived.

Probably, FF cells may contribute to the competence and the fate of the oocyte and play an important role within the oocyte micro-environment [28]. If these data were confirmed the association between FF endocrine pattern *in vitro* and pregnancy let us to believe that FF cells may be a useful model to investigate prognostic factors in IVF procedures.

FF cells were obtained in a simple and cheap manner during routine IVF procedures and were isolated from follicular fluid that is usually wasted after cumulus-complex-oocyte retrievial.

Numerous recent findings showed that FF cells were extremely versatile. In fact these cells express typical mesenchymal markers (CD90, CD44, CD105, CD73 and vimentin), had a stem cell behaviour because of their clonogenic capacity and multipotency but also preserve granulosa aspects. They can be cultured without any growth factor in order to reduce cost management and became useful to many kind of applications in biological research. According to this finding, it is tentatively suggested that this fluid may become an alternative source for MSC and may be worth conserving after IVF procedures. The characteristics of follicular fluid suggest the possible use of follicular fluid cells as cheap biological experimental model for basic research in stem cells, in the field of tissue engineering and in the basic studies on human reproductive medicine.

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Austin Therapeutics - Volume 1 Issue 2 - 2014
ISSN: 2472-3673 | www.austinpublishinggroup.com

Citation: Omes C and Riva F. New Experimental Model for Basic Research in Stem Cell Field. Austin Therapeutics. 2014;1(2): 3.