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RESEARCH ARTICLE

Effect of Pulsed Electromagnetic Fields on Human Osteoblast Cultures

Q1 Simona Barnaba[1], Rocco Papalia[1], Laura Ruzzini[1], Alessandro Sgambato[2], Nicola Maffulli[3]* & Vincenzo Denaro MD[1]

[1]Department of Orthopaedic and Trauma Surgery, Campus Biomedico University of Rome, Via Alvaro del Portillo 200, Rome, Italy

[2]Giovanni XXIII Cancer Research Center-Institute of General Pathology, Catholic University, School of Medicine, Largo Agostino Gemelli, 8, Rome, Italy

[3]The Centre for Sports and Exercise Medicine Barts and The London School of Medicine and Dentistry, Mile End Hospital, London, UK

Abstract

Background and Purpose. Exogenous electromagnetic fields (EMFs) affect bone metabolism, but the mechanisms responsible for this phenomenon are unclear. Pulsed EMFs (PEMFs) can be effective in the management of congenital pseudarthrosis or delayed union or non-union of fractures. We investigated the effects of PEMFs used in clinical practice on human osteoblast cultures. **Methods.** Primary osteoblastic cells were isolated from a human femoral head. Cultures were exposed to the PEMF stimulation for 72 hours, 7 and 10 days and compared with a control group of primary osteoblastic cells non-exposed to PEMF. Cell growth and alkaline phosphatase activity were evaluated in the osteoblast cell cultures at each observation time. **Results.** At each observation time, the differences in cell numbers between Q2 PEMF-exposed cells and control group were statistically significant ($p < 0.05$). The alkaline phosphatase-specific activity of PEMF-exposed osteoblast cultures showed a statistically significant ($p < 0.05$) increase when compared with the control group after 7 and 10 days of exposure. **Conclusions.** The application of PEMF stimulation on human osteoblasts accelerates cellular proliferation when compared with a control group of non-PEMF-exposed cells. Copyright © 2012 John Wiley & Sons, Ltd.

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Keywords

biophysical stimulation; bone cells; cell cultures; cell proliferation

***Correspondence**

Nicola Maffulli, Centre Lead and Professor of Sports and Exercise Medicine, Consultant Trauma and Orthopaedic Surgeon, Centre for Sports and Exercise Medicine, Barts and The London School of Medicine and Dentistry, Mile End Hospital, 275 Bancroft Road, London E1 4DG, England. E-mail: n.maffulli@qmul.ac.uk

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Q3 Introduction

After the experiments of Yasuda et al. (1955) on the effect of electrical stimulation on bone metabolism, electrically induced osteogenesis has been studied intensely both *in vivo* and *in vitro* (Spadaro, 1997, Yasuda et al., 1955). Despite clinical success, the mechanism by which electrically induced osteogenesis occurs remains partially unexplained, and the data regarding the effect of both pulsed electromagnetic fields (PEMFs) and static magnetic fields (SMF) on osteoblast proliferation and differentiation have been contradictory.

Biophysical inputs, including electric (EF) and electromagnetic fields (EMFs), regulate the expression of genes for structural extracellular matrix (ECM)

proteins resulting in acceleration in tissue repair. EF and EMFs can increase the synthesis of growth factors through activation of cell signal transductions, enhancing in this way endochondral bone formation (Aaron et al., 2004).

Electromagnetic fields affect different aspects of biomolecular synthesis in cells, including the kinetics of DNA, RNA and protein production (Liboff et al., 1984). Increased DNA and proteoglycan synthesis has been observed in chondrocytes, whereas fibroblasts showed altered collagen and proteoglycan synthesis (Farndale and Murray, 1985, Lee and Pelker, 1985).

There are three different methods of EF/EMF bone growth stimulation: capacitive coupling using electrodes placed on the skin, direct current stimulation using implanted electrodes and electromagnetic stimulation by inductive coupling using time-varying magnetic fields. Clinical application of the latter category is possible through two different Food and Drug Administration (FDA)-approved technologies: PEMF and combined magnetic fields (Pilla, 2002). The use of PEMF was approved by the FDA in 1979 and has been used clinically for over 26 years. Initially, this form of athermal energy was used as a salvage option for patients with long-standing bone non-unions resistant to conventional forms of surgical treatment. PEMF can promote healing of acute fractures, delayed union and non-union of fractures, congenital pseudarthosis and failed arthrodesis (Taylor et al., 2006). PEMF appears to affect already differentiated bone cells through various transduction pathways and growth factors, decreasing osteoclastic resorption and increasing osteoblastic bone formation (Taylor et al., 2006). Studies using bone cell cultures showed that electromagnetic stimulation with PEMF promoted DNA synthesis within the osteoblasts, suggesting in this way a probable influence of EMF on cell nuclear mechanisms (Goodman et al., 1985, Korenstein et al., 1984).

Static magnetic fields are a different type of EMF mainly used in dentistry (Darendeliler et al., 1995, Riley et al., 2001) Although there are studies supporting that PEMF are more effective in stimulating bone metabolism (McLeod and Rubin, 1992), using SMF could be advantageous, especially in long-term therapy, as SMF does not need an external energy source when a permanent magnet is used. Studies on animals demonstrated that long-term applications of SMF favour bone mineral density (Bruce et al., 1987) However, *in vivo* studies of SMF applied on periprosthetic bone demonstrated that SMF induces the production of corrosion currents inhibiting osteoblasts differentiation pattern and decreasing bone mineral density (Denaro et al., 2008a).

We report the results of the exposure of human osteoblast cultures to PEMFs of the intensity used in clinical practice. Our null hypothesis was that EMFs of different kind and intensity did not differently affect the same osteoblast cell cultures.

Materials and methods

Electromagnetic field product

The exposure system was composed of a waveform generator and a stimulation coil. The stimulation coil was a 30-cm-long solenoid that is 15 cm in diameter, with 45 turns of 20-gauge magnet wire. A pulsed EMF of 0.4 mT with a frequency of 14.9 Hz was produced by an FDA-approved machine for the treatment of non-union or delayed union of bone fractures. The magnetic field was directed parallel to the coil axis and was uniform inside the coil. The coil support was constituted by copper wire that can survive intact for days in an incubator at 100% relative humidity and 37 °C.

Cell cultures

Primary osteoblastic cells were isolated from human femoral heads obtained from healthy subjects who underwent hip replacement surgery for osteoarthritis. Three sequential digestions of 20, 40 and 60 minutes, respectively, were performed with type IV collagenase and 0.25% trypsin in Hank's buffer solution. Cells from the latter digestion were plated and cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich Co, St Louis, MO) supplemented with 1% penicillin-streptomycin (Pen-strep) (Sigma-Aldrich Co, St Louis, MO) and 10% fetal bovine serum (Sigma-Aldrich Co, St Louis, MO) at 37 °C in a humidified atmosphere of 5% CO_2 in air. At confluence, cells were trypsinized and amplified for characterization and to be used for these studies. Only cells from the second and third passages were used for the experiments.

Cell growth analysis

Two distinct cell cultures were performed, one PEMF exposed and the other non-PEMF exposed (control group).

Control and PEMF-exposed cells were incubated separately inside two identical incubators (one for

control cells and one for PEMF-exposed cells). Cultures were exposed to PEMF stimulation for 72 hours, 7 and 10 days.

Cell growth was expressed as cells/mL. At each observation time, the cell monolayers were washed with phosphate-buffered saline (PBS) (Sigma-Aldrich Co, St Louis, MO) and detached with 0.25% trypsin/ethylenediaminetetraacetic acid (Sigma-Aldrich Co, St Louis, MO) for 10 minutes at 37 °C. Cell suspensions were centrifuged at 1200 rpm for 10 minutes at 25 °C, and the supernatant was discarded. The pellet was re-suspended in PBS, and cells were counted using an automated cell counter.

All assays were performed in triplicate. A total of nine assays were performed (three for each observation time) for both PEMF-exposed and control cells.

Alkaline phosphatase activity

Cells were fixed in 3% paraformaldehyde in 0.1-M cacodylate buffer for 15 minutes and washed in the same buffer. Alkaline phosphatase (ALP) activity was detected histochemically using Sigma-Aldrich kit 85, according to the manufacturer's instruction.

Alkaline phosphatase activity in the cell cultures was quantified after 72 hours, 7 and 10 days of PEMF exposure, as the rate of conversion of *p*-nitrophenyl phosphate to *p*-nitrophenol at a pH of 10.2.

Briefly, cells were cultured in 3.5 and 10-cm Petri dishes. At each observation time, the medium was aspirated, the cells were washed twice with PBS, at 500 µL and 1 mL 0.05%, respectively, and Triton X-100 (Sigma-Aldrich Co, St Louis, MO) was added to each dish. After cell disruption by three freeze/thaw cycles, 50-µL cell lysate from each dish was transferred to a 96-well microliter plate. Absorbance was measured at 405 nm. Enzyme activity was evaluated for 60 minutes and expressed as Vmax. Reported values were normalized against protein concentration determined in total cell lysate.

Statistical analysis

Data are typical results from three replicated experiments and are expressed as mean ± standard deviation. The intraclass correlation coefficient was used to validate the reliability of the cell counter. Comparisons between PEMF and control (unexposed) groups were performed using paired Student's *t*-test; p values less than 0.05 were considered significant.

Results

The analysis of the three cell counter measurements obtained for each cell assay revealed a high intraclass correlation coefficient ($r = 0.975$; $p < 0.05$).

Proliferation rate

At each observation time, differences in cell numbers between PEMF-exposed cells and control group were statistically significant ($p < 0.05$).

Pulsed EMF-exposed osteoblasts showed an increased proliferation rate compared with untreated control cells. Differences in cell numbers between control and PEMF-exposed cells were statistically significant ($p < 0.05$) after 7 and 10 days of exposure: the PEMF group showed a 1.8% ($p > 0.05$), 29% and 55.5% increase in cell number after 72 hours, 7 and 10 days, respectively.

Alkaline phosphatase activity

An increase of ALP staining was detected in PEMF-exposed osteoblast cultures compared with control cultures. The ALP-specific activity of PEMF-exposed osteoblast cultures was significantly ($p < 0.05$) increased when compared with the untreated control group after 7 and 10 days of exposure. The PEMF group showed a 1% ($p > 0.05$), 20% and 58% increase of ALP activity after 72 hours, 7 and 10 days, respectively (Figure 1).

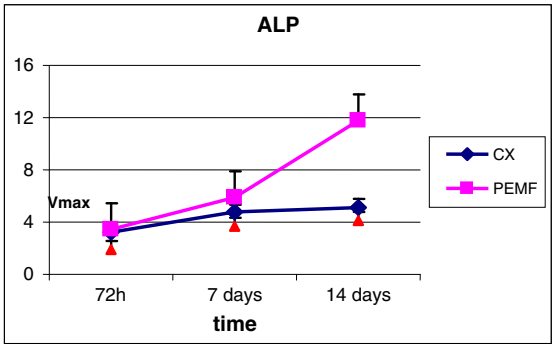

Figure 1. The chart shows that the alkaline phosphatase (ALP)-specific activity pulsed electromagnetic field (PEMF)-exposed osteoblasts showed an increase in ALP activity compared with the control (CX) untreated cells. Data shown represent the mean (± standard error) of three independent experiments, each yielding similar results. $p < 0.05$

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Discussion

Pulsed EMFs can be effective in the management of congenital pseudarthrosis or delayed union or non-union of fractures (Trock, 2000). Exogenous EMFs affect bone metabolism, but the mechanisms responsible for this phenomenon are unclear. PEMFs can affect bone turnover, decreasing osteoclastic resorption and increasing osteoblastic bone formation through activation of various transduction pathways and growth factors (Chang et al., 2004, Fitzsimmons et al., 1995, Otter et al., 1998, Ryaby, 1998).

Pulsed EMFs induce the upregulation of several genes related to the formation of bone and matrix components, and stimulate downregulation of genes related to the degradation of ECM (Sollazzo et al., 2010). Moreover, PEMF can directly stimulate bone marrow-derived stromal cell towards osteogenic differentiation (Jansen et al., 2010, Tsai et al., 2009, Lee et al., 1997), and we have previously demonstrated that PEMFs comparable with the ones used for the management of pseudarthrosis could stimulate closure of an *in vitro* wounding of a tenocyte monolayer(Denaro et al., 2010).

According to some authors, PEMF stimulation can significantly increase osteoblasts proliferation but does not affect cellular differentiation (Chang et al., 2004). In contrast, Diniz et al. (2002) showed that PEMF had a stimulatory effect on the osteoblasts in the early stages of culture, leading to an increased bone tissue-like formation because of the enhancement of cellular differentiation.

In vitro, PEMF can influence osteoblasts by increasing the basal level of intracellular [Ca2+], ECM production, IGF-II and TGF-β secretion and by decreasing PGE2 secretion, enhancing the sequence of events leading to bone tissue formation(Fitzsimmons et al., 1995, Lohmann et al., 2000, Taylor et al., 2006). PEMF can also influence *in vitro* osteoblasts morphology and orientation (Lee and McLeod, 2000). PEMF-exposed osteoblast-like cells are consistently smaller than sham-treated cells and are oriented orthogonal to the applied magnetic field, showing significant decrease in cell length and increase in roundness (Lee and McLeod, 2000), suggesting an influence on cell differentiation.

Static magnetic fields are another type of EMF used in clinical practices, especially dentistry. *In vitro* studies have shown that PEMF are more effective on bone formation than SMF, and moderate-intensity SMFs ranging from 1 mT to 1 T influence a wide variety of biological systems (Okano et al., 2006, Lee and McLeod, 2000, Huang et al., 2006). Continuous SMF (Huang et al., 2006) induce osteoblastic cell differentiation at an early stage; these effects might be achieved by regulating early local factors released by the cells.

Low-intensity SMFs produced by spinal metal devices lead to a downregulation of both osteoblast proliferation and differentiation, confirming that EMF generated by spine titanium implant can be linked to peri-implant aseptic osteolysis (Denaro et al., 2008a, Denaro et al., 2008b).

The effect of different type of EMFs (SMF and PEMF) on cells of bone lineage is controversial, and reports about the effects of PEMF and SMF stimulation on osteoblast proliferation and differentiation have been contradictory.

Comparing the present results with our previous results (Denaro et al., 2008a) on the effect of SMF on the same cell cultures, we found that SMFs comparable with the one produced around metal devices have different effects leading to inhibition of osteoblast ALP-specific activity. In practice, PEMF produce upregulation of osteoblast proliferation and ALP activity, whereas SMF produce downregulation of the same variables, accounting for the osteolytic effects of the EMFs generated by titanium vertebral implant (Denaro et al., 2008a).

Comparing the results of the present investigation with the published evidence, we found that the maximal difference in the ALP activity between the SMF-exposed cells and control was after 72 hours of stimulation, whereas in PEMF-exposed cells, proliferation and differentiation are influenced after more than 72 hours of exposure (Figure 2). The interaction of EMFs with a biological system must include activation of a cellular

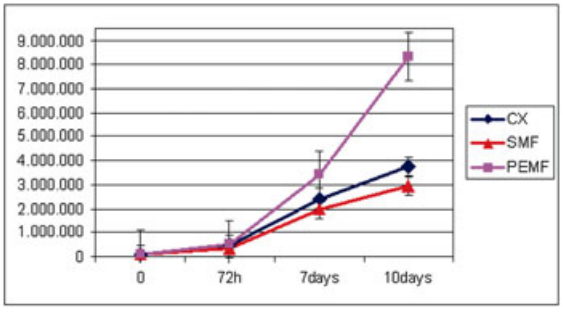

Figure 2. The chart shows the osteoblasts cell growth. Differences in cell numbers between pulsed electromagnetic field (PEMF)-exposed, static magnetic field (SMF)-exposed and control (CX) groups were statistically significant at each observation time. Data shown represent the mean (± standard deviation) of three independent experiments, each yielding similar results

process and of signal transduction pathways (Simko and Mattsson, 2004).

It is also possible that *in vitro* response of the osteoblasts to EMFs depends on EMF features such as frequency and intensity that probably influence cellular processes at different stages of differentiation.

It is difficult to determine whether differences in responses to biophysical factors arise from the type of signal or the form or duration of the signal (Schwartz et al., 2008). The mechanisms underlying these effects on proliferation and differentiation produced by EMFs with different features need to be clarified.

The effects of EMF on human osteoblast cultures appear to be dependent on differentiation stage: SMF-exposed cultures show maximal difference in the ALP activity after 72 hours of stimulation, whereas PEMF-exposed cells show that proliferation and differentiation are influenced after more than 72 hours of exposure.

Conclusion

Different types of EMFs could differently influence the molecular mechanism of stimulation of bone cells activities. The application of different types of EMFs determines different effects on human osteoblasts. PEMF stimulation accelerated cellular proliferation and differentiation, whereas SMF-exposed cells show a decrease of both proliferation rate and differentiation (Denaro et al., 2008a).

Conflict of interest

We declare no conflict of interest and no grant of financial profit related to our study.

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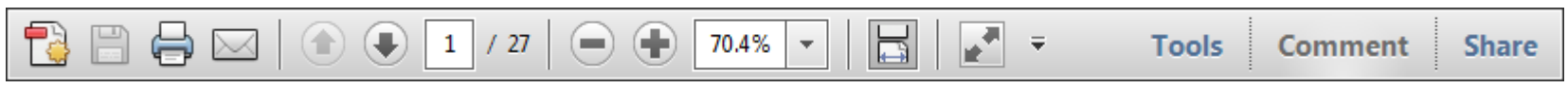

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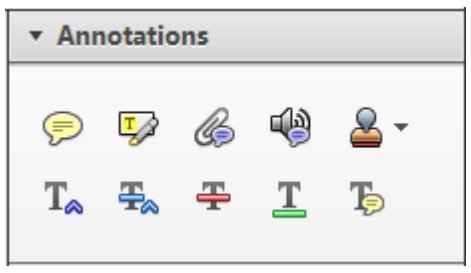

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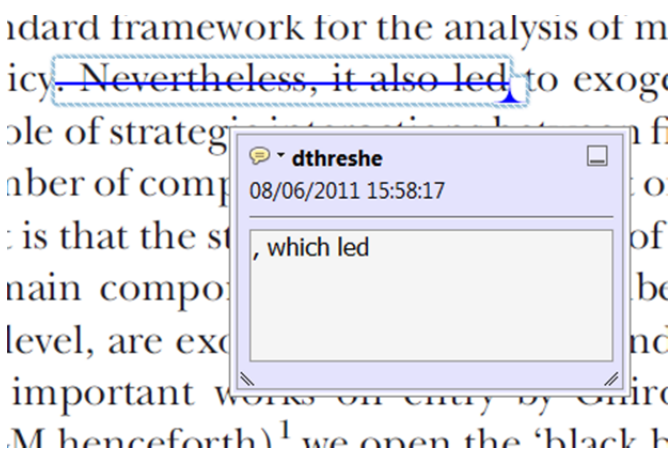

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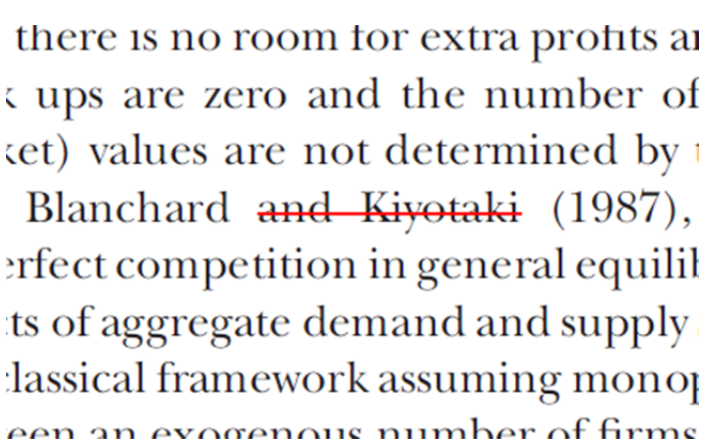

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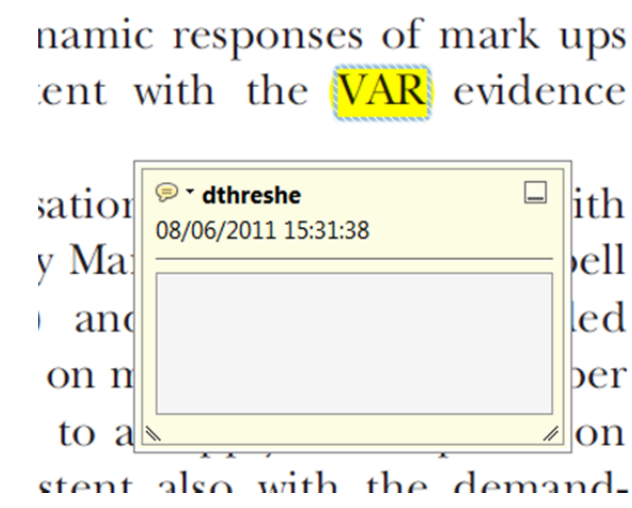

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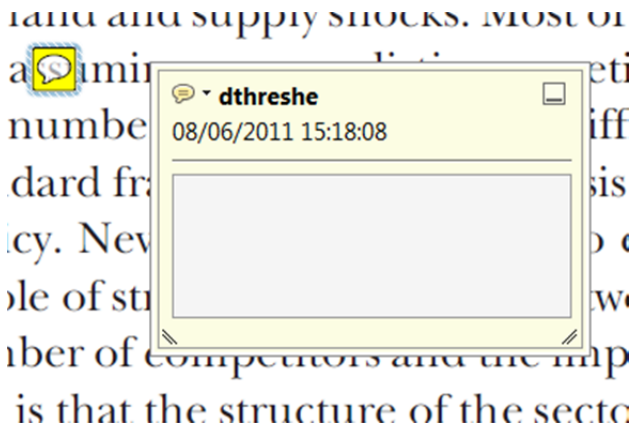

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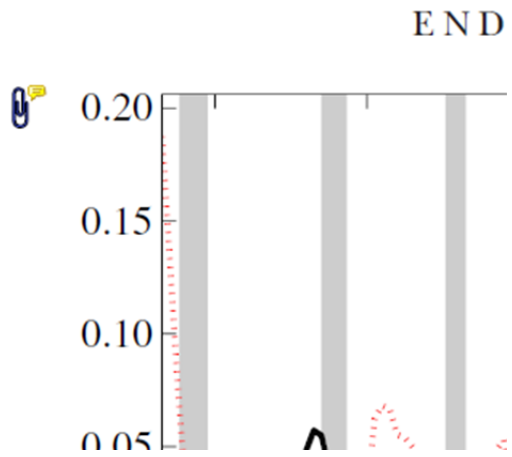
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

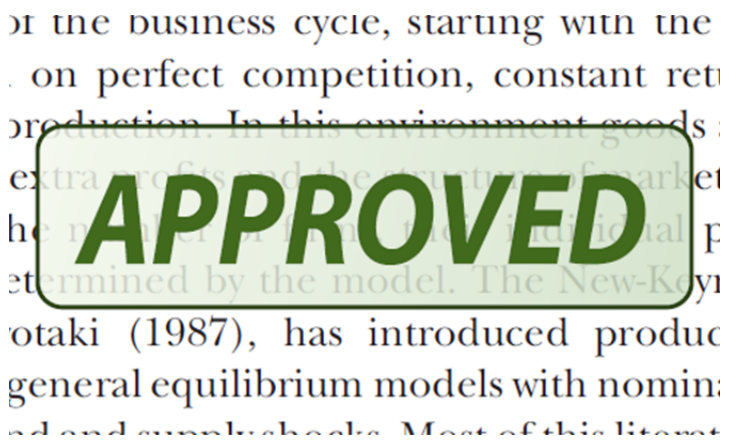


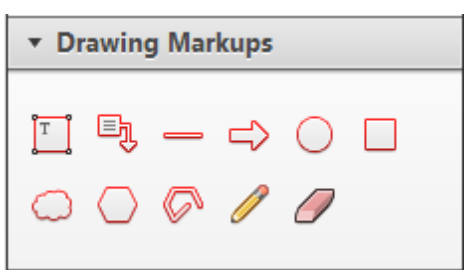
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).



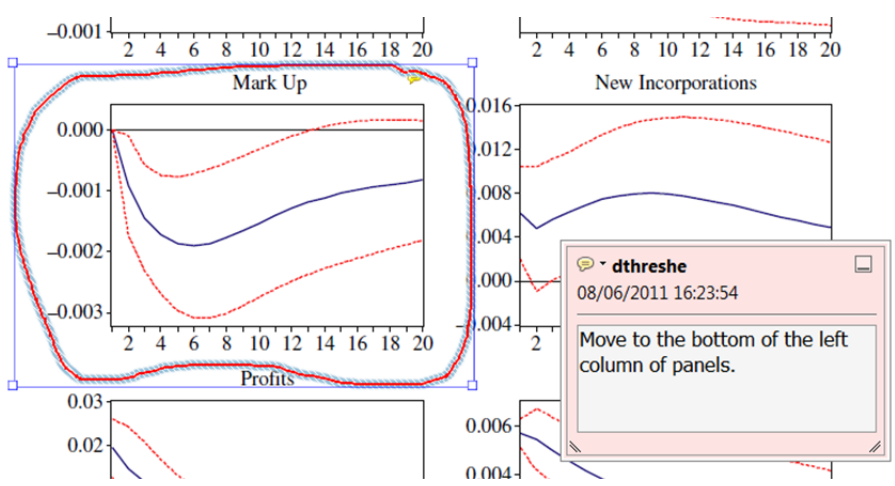


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: