



# The Influence of MgO Nanoparticles on the Osseointegration of Polycaprolactone - Sodium Alginate Hydrogel Interfaces

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## Authors' contributions

*This work was carried out in collaboration between all authors. Authors MK and BS designed the study and supervised the experiments. Author MBV directed the absorbance test study. Author MK wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The aims of this study were to evaluate the influence of magnesium oxide (MgO) nanoparticles on the cell viability of a sodium alginate (SA) hydrogel and to evaluate the influence of MgO nanoparticles on the interface tensile strength between polycaprolactone (PCL) and SA hydrogel scaffolds after two weeks of cell culture.

**Study Design:** Mouse osteoblast cells (MT3T3E1) were cultured on two groups of scaffolds: SA hydrogel and SA hydrogel with 22 nm MgO particles. Quantitative cell viability tests were conducted on each of the samples to compare the influence of magnesium oxide (MgO) nanoparticles on cell viability between the two groups. MT3T3E1 cells were cultured on two groups of coupled PCL-SA hydrogel scaffolds: PCL-SA hydrogel scaffold and PCL-SA hydrogel scaffold with 22 nm MgO particles. Tension tests were conducted on the coupled samples to compare the interface tensile strength between the two groups.

**Place and Duration of Study:** The studies were carried out in the Stephenson Research and Technology Center at University of Oklahoma, the Interface Tissue Engineering Laboratory at the University of Central Oklahoma, and the Cell Biology Research

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Laboratory at the University of Central Oklahoma between June 2009 and May 2011.

**Methodology:** Standard cell culture protocols were used to culture cells on SA hydrogel scaffolds with and without MgO nanoparticles for 4 and 24 hours. Absorbance and fluorescent intensity tests were conducted for quantitative measurements of cell viability. Cells were cultured on PCL-SA coupled scaffolds for 2 weeks. A custom tension setup was designed and fabricated to conduct tension tests on the coupled scaffolds to quantify the mechanical strength of the osseointegration.

**Results:** This research found that SA hydrogel scaffolds containing MgO nanoparticles demonstrated higher osteoblast cell activity compared to SA hydrogel without MgO. The study also found reduced interface tensile strength when PCL-SA coupled scaffolds contained MgO nanoparticles.

**Conclusion:** This study thus suggested that MgO nanoparticle improves the cell viability of SA hydrogel, but it is detrimental for the osseointegration of PCL-SA hydrogel constructs.

*Keywords: Tissue engineering; polycaprolactone; sodium alginate; magnesium oxide; osseointegration.*

## 1. INTRODUCTION

Integration of engineered tissue constructs within the body is a long-standing problem in medicine and tissue engineering. Although most tissue engineering research has focused on interface formation, the tissue-implant interface generated by the host tissue should maintain stability under functional loading [1]. The success of tissue reconstruction efforts requires a thorough understanding of the structure-function relationship existing at the native insertion site of the grafted tissue [2]. There has not been much research done on the ability of nanomedicine to improve the interface strength of tissue-biomaterial [3]. Gulotta et al. [4], using a rabbit anterior cruciate ligament model, found that magnesium-based bone adhesive improves tendon-to-bone healing at 6 weeks based on histologic and biomechanical testing. Soft engineering tissues such as hydrogel are able to integrate with hard tissue; however, a challenge remains to improve grafts between hard tissues and soft tissues. The interface between native tissue and engineered tissue as well as the interface between different engineered tissues is the weakest place in the tissue engineered constructs. A strong osseointegration of these tissue surfaces at the interface is important for the success of engineered tissue constructs. Nanomedicines such as nanoparticles and nanofibers can be used to enhance osseointegration between two different engineered tissue constructs. The question arises whether MgO nanoparticles can be used as nanomedicine to improve the osseointegration of an engineered hard – soft tissue construct. The hard tissue construct studied in this research was a three dimensional polycaprolactone (PCL) scaffold. The soft tissue construct studied in this research was a sodium alginate (SA) hydrogel.

The osseointegration between hard tissue and soft tissue can be created by cell culturing on coupled PCL-SA hydrogel constructs. The polycaprolactone (PCL) hard tissue graft is an effective bone scaffold because it has properties that allow for successful cell adhesion. The PCL based 3D scaffold is an effective scaffold because it allows for cell spreading, migration, viability, and growth [5]. PCL is made using a precision deposition system which allows for the scaffold to have precise pore sizes and porosity, which promotes increased cell adhesion [6,7]. Hydrogel scaffolds are engineered to resemble the mechanical properties of cartilage or ligament tissues in the body [8]. SA hydrogel, made of a hydrated polymer gel, has been used as a tissue engineering scaffold for soft tissue grafting [9]. SA hydrogel can effectively be made from sodium alginate and a calcium chloride solution [10]

that, when combined, becomes a soft clear gel over time. The hydrogel is an effective scaffold because it is a porous structure which allows the cells to grow [11]. Hydrogel containing cells can be introduced into the body to replace cartilage and help the body heal [12]. Both PCL and SA scaffolds are biodegradable and biocompatible in the human body and can be used for bone-ligament or bone-cartilage reconstructions. Our study found that MgO is one of the most suitable additives to PMMA, since osteoblast cell adhesion to the MgO-included PMMA specimens was significantly higher than the cell adhesion to PMMA cement-only specimens ( $P < 0.001$ ) and no significant change of mechanical strength was observed due to the addition of MgO to PMMA [13]. Ricker et al. [14] demonstrated that compared to micro particles, PMMA with nanoparticles of MgO reduced harmful exothermic reactions of PMMA during solidification, increased radiopacity and increased osteoblast cell function. Therefore, the nanosize MgO particle is a better choice as medicine for biomedical applications compared to the microsize MgO particle. The influence of MgO nanoparticles on the cell-dependent mechanical integration of PCL and SA tissue constructs has not been investigated. The motivation of this study was to evaluate the MgO nanomedicine effect on the osseointegration of PCL-SA hydrogel constructs.

This study hypothesized that the MgO nanoparticles could improve the cell-dependent mechanical integration of hydrogel and PCL tissue surfaces. Quantitative cell viability tests were conducted on SA hydrogel with MgO to examine the effect of MgO concentration on osteoblast cell viability within the hydrogel scaffold. Tensile tests were conducted on coupled PCL- SA hydrogel and PCL- SA hydrogel with MgO nanoparticles samples, which were cell cultured for 2 weeks. A custom made tensile stage was fabricated to measure the interface adhesive strength of these PCL-SA constructs at the end of the cell culture period.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

The hard tissue construct studied in this research was a three dimensional polycaprolactone (PCL) scaffold. 3D Insert-PCL scaffolds (scaffold diameter: ~21 mm Scaffold height: ~1.5 mm Fiber Diameter: ~300 microns Spacing: ~300 micron) were purchased from 3D Biotek (Hillsborough, NJ). The soft tissue construct studied in this research was sodium alginate (SA) hydrogel. Protanal® LFR 5/60 sodium Alginate was purchased from FMC BioPolymer (Philadelphia, PA). Sodium Alginate was mixed with Dulbecco's Modified Eagle Medium (DMEM) to prepare a 5% sodium alginate solution. MgO particles were purchased from Sigma-Aldrich. Particle size distributions were measured by Microtrac Inc. (Montgomeryville, PA). The results of nanoparticle size distribution were reported in Khandaker et al. [15]. This previous study confirmed that the sizes of MgO particles are in the ranges of nanometers.

### **2.2 Cell Culture**

Mouse osteoblast cells (MT3T3E1) were obtained from the American Type Culture Collection (ATCC). Cells were cultured in a flask before seeding to SA hydrogel according to ATCC protocols [16]. Cells were cultured in a medium consisting of Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were incubated at 37°C in a 5% CO<sub>2</sub> humidified atmosphere. The medium was refreshed approximately every three days. Prior to refreshing the medium, cells were washed with Dulbecco's Phosphate-Buffered Saline (PBS) to remove dead cells. Once becoming confluent, cells were split into separate flasks. Trypsin was used to detach the

cells from the flask wall. The cells and trypsin were placed in a centrifuge for 5 minutes at 1500 RPM, to form a small pellet of cells at the bottom of the centrifuge tube. The trypsin was removed and the pellet was resuspended with medium. The medium, containing cells, was dispensed into two separate flasks for seeding hydrogel and PCL scaffolds. Cells were also seeded into the hydrogel and hydrogel with MgO particles at a cell density of 250k/ml. The cells were removed from the culture flask using the same method as splitting cells. Instead of separating the cells into two separate flasks, the medium was added to a 5% sodium alginate solution and sufficiently mixed. Enough medium was added so that the 5% sodium alginate solution decreased to 4%. 200 $\mu$ l of 5% CaCl<sub>2</sub> solution was placed in a well plate, followed by 1000 $\mu$ l of 4% sodium alginate mixed with medium containing cells, at a 1 to 5 ratio, respectively. The sodium alginate and calcium chloride solutions were sterilized in an autoclave. After 2 hours fresh medium was placed on top of the hydrogel and incubated.

### **2.3 Cell Viability Tests**

Mouse osteoblast cells (MT3T3E1) were cultured on PCL scaffold, and SA hydrogel scaffold in the presence or absence of 22 nm MgO particles. Cell viability was determined in each hydrogel after 4 and 24 hrs. The rationale for conducting 4 and 24 hours of cell culture was to verify the positive effect of cell culture time on the cell viability of hydrogel samples. The viability testing of cells was conducted quantitatively using absorbance tests and fluorescent intensity tests. Absorbance tests were conducted for determining the appropriate concentration of MgO nanoparticles. Cells were cultured in cell culture flask (control) and hydrogel with different concentrations of MgO nanoparticles (0 wt%, 0.1 wt%, 0.5 wt%, 1 wt%, 2 wt%). Absorbance tests were conducted using Multiskan EX photometric microplate absorbance reader (Thermo Electron Corporation, Vantaa, Finland). Photometric absorbance readings at 450nm were measured for all samples. Fluorescent intensity tests were conducted on hydrogel with cells and hydrogel with the appropriate concentration of MgO nanoparticles using Fluostar Optima Microplate Reader (BMG Labtech) for determining the quantitative measurement of cell viability on hydrogel samples. Alamar Blue was used to permit fluorophore loading on hydrogel. Alamar blue is initially a blue color and will change to red if the cells are active in their environment. The fluorescence intensity, which indicates the amount of fluorophore loading on hydrogel, was directly measured from the microplate reader. The amount of fluorophore loading quantifies the level of cell activity on hydrogel samples [17]. The cells' activity on different hydrogel samples was compared by averaging the fluorescent intensity values.

### **2.4 PCL-SA Hydrogel Specimen Preparation**

Two different types of PCL-hydrogel tissue constructs were tested: PCL-hydrogel and PCL-hydrogel with 0.1 wt% of MgO nanoparticles. The 5% sodium alginate was added to 5% CaCl<sub>2</sub> to make hydrogel scaffolds (height ~1.5 mm, scaffold diameter~21 mm) in a sylgard mold for PCL-SA hydrogel constructs. Osteoblast cells were cultured on hydrogel and hydrogel with MgO nanoparticles scaffolds according to ATCC instructions. The hydrogel scaffolds were separated from sylgard mold and carefully placed on top of the PCL. After placing the hydrogel and PCL together, 1 ml of medium was added on top and refreshed daily. To increase the interfacial bond between the PCL and hydrogel constructs, cells were cultured on the PCL scaffolds according to 3D Biotek protocols [18]. Cells were cultured in PCL in a 12-well cell culture plate using the same cell density (250k/ml.). The hydrogel was placed on top of the PCL to form an interfacial bond between the two scaffolds. Approximately 3.9 Pascals of pressure was added on top using a sterilized aluminum plate,

in order to help increase the interfacial bond. The PCL-SA constructs were cultured for 2 weeks. Approximately every two days the medium was refreshed.

## 2.5 Design and Manufacture of the Experimental Setup

A custom made tension stage (Fig. 1) was designed and fabricated for the tensile experiment of the PCL-hydrogel samples. The test stage consisted of two specimen holders. One holder was fastened to the setup base. The other specimen holder was connected with a 250 gram load cell (Futek™ LCM300, model number FSH02630) with a sensor (Futek™ IPM500). The other end of the loadcell was connected with a high precision microactuator (Newport™ LTA-HL®) and motion controller (SMC 100). A xyz stage was assembled with the test stage for microscopic viewing purposes of the samples. All instruments were calibrated before testing. The PCL-hydrogel samples were carefully glued to the specimen holders during the tension test.

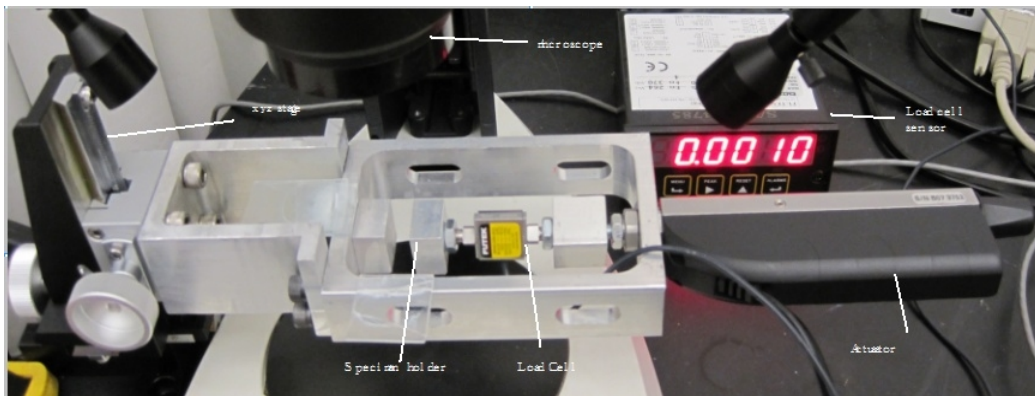
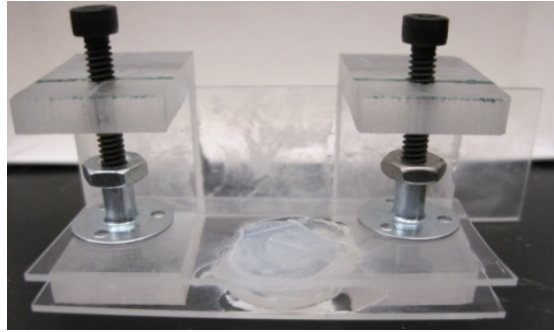


Fig. 1. Experimental setup used for tension tests on PCL-SA hydrogel samples

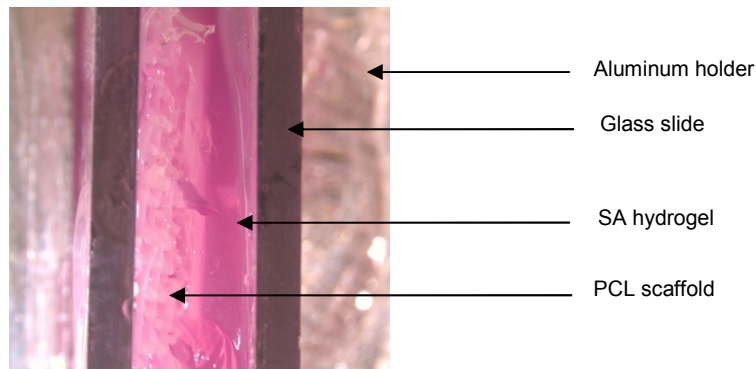
## 2.6 Tensile Tests on PCL-SA Hydrogel Samples

In order to prevent damaging the interfacial bond of the hydrogel and PCL tissue construct, a clamp was used to hold the structure in place while gluing it into the mechanical testing device. Two clamps were built and then glued together, on a 2.25 inch long glass plate, which could easily slide into the mechanical testing device (Fig. 1). Epoxy was used to attach the tissue construct to glass plates. In order to ensure better adhesion of the glass plate to the hydrogel, the top of the hydrogel was shaved to create a smooth surface. After attaching with epoxy, the specimens were placed in the incubator for thirty minutes to allow for proper adhesion. Then, Loctite 411 glue was used to attach the glass plates into the opening of the mechanical testing device. This glue dried within a few minutes. The PCL-hydrogel specimens as shown in

Fig. 3 were tested under moist conditions using DMEM solution. The loading rate was 0.001 mm/s. The load and displacement data were continuously recorded until the failure of the PCL-hydrogel joint. The load and displacement data were recorded and analyzed to determine the maximum apparent interface tensile strength,  $\sigma_f$ , using the relationship [19]:  $\sigma_f = P_{cr}/A$ , where  $P_{cr}$  is the maximum value of the load-displacement curve and  $A$  is the cross-sectional area of interface, which is calculated using the equation:  $A = \pi d^2/4$ , where  $d$  is the diameter of the interface area.



**Fig. 2. PCL-SA hydrogel specimen in a custom made clamp before the testing**



**Fig. 3. A PCL-hydrogel sample for tension test**

### **3. RESULTS AND DISCUSSION**

#### **3.1 Cell Viability Tests**

Absorbance tests were conducted ( Fig. 4) to determine an adequate amount of MgO nanoparticles to add to the hydrogel to increase adhesive strength, while not decomposing the hydrogel itself. The 0.5% and 0.1% were most closely compatible with the hydrogel with cells, so it was determined that the 0.1% would be sufficient to use in the hydrogel.

Fig. 5 compares the fluorescence intensity of hydrogel with and without 22 nm MgO particles after 4 and 24 hours of cell culture. As expected, higher fluorescence intensity was found for both hydrogel samples after 24 hours of cell culture compared to the fluorescence intensity of both samples after 4 hours of cell culture. The test results also show that the average fluorescence intensity of hydrogel with MgO nanoparticles is higher than without MgO nanoparticles for both 4 and 24 hours of cell culture periods. The difference of fluorescence intensity between hydrogel samples was statistically significant after 24 hours of cell culture (P value = 0.003) but not after 4 hours of cell culture (P value = 0.71). This result suggested that MgO nanoparticles increase the cell viability in hydrogel, but the increase was not noticeable after 4 hours of cell culture.

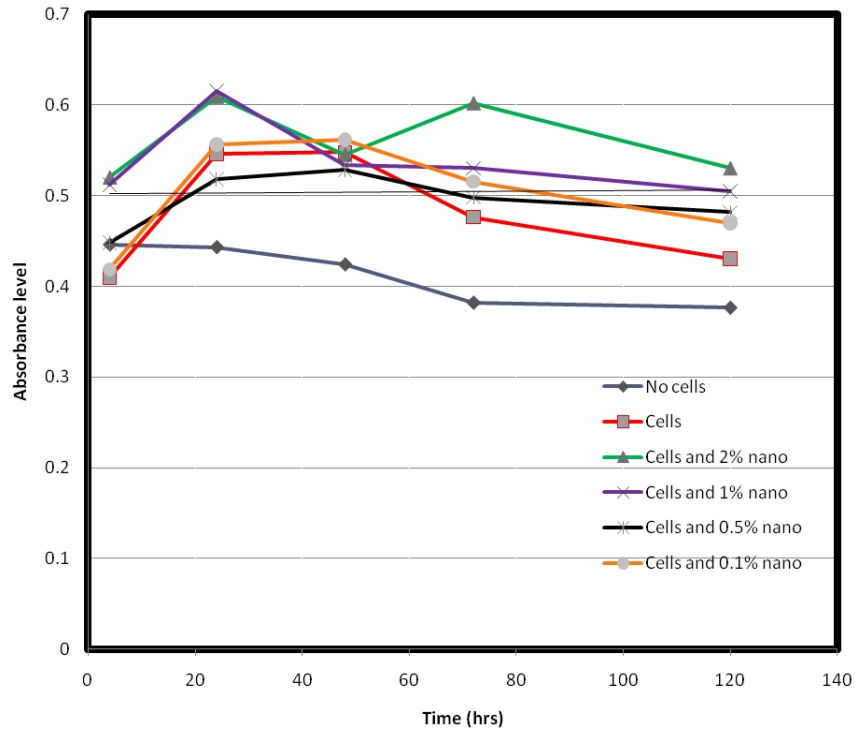


Fig. 4. Absorbance test on cell seeded hydrogel with different concentrations of MgO.

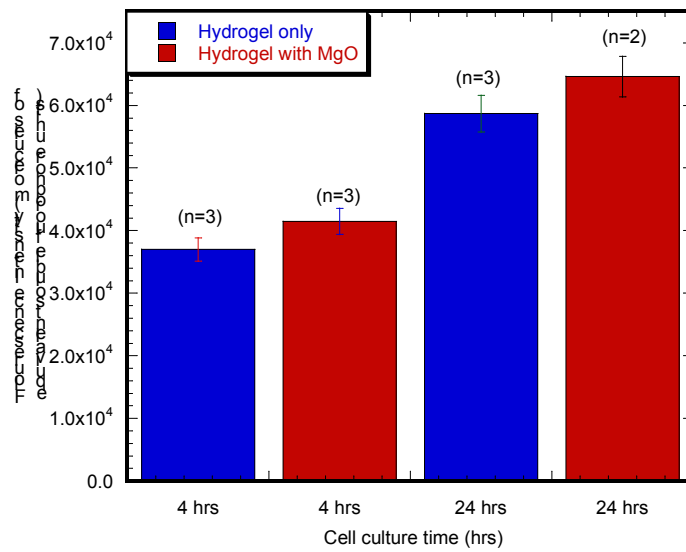


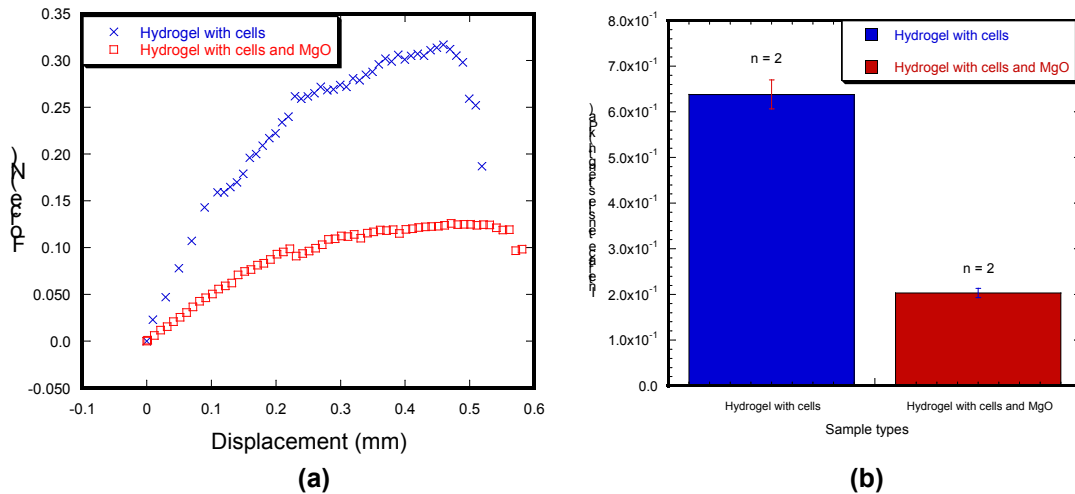
Fig. 5. Fluorescence intensity tests on hydrogel with and without 22 nm MgO particles after 4 and 24 hours of cell culture

### 3.2 Tension Tests

Fig. 6(a) compares the load-displacement curves of PCL-hydrogel with and without 22 nm MgO cultured with cells for two weeks. The load-displacement response of all specimens was characterized as an elastic response initially, followed by a long inelastic region and then a stable descending response. Fig. 6(b) compares the interface tensile strength values. Results show decreased interface tensile strength between cell-seeded PCL-SA hydrogel mixed with MgO nanoparticles. The interface strength of PCL-SA hydrogel without nanoparticles was higher than PCL-SA hydrogel with MgO nanoparticles.

This result is due to the fact that SA hydrogel containing MgO nanoparticles was disintegrated and became more porous after two weeks of cell culture. The increased porosity led to a cell number increase but an interface surface area decrease for PCL-SA hydrogel with MgO nanoparticles. This explanation is in agreement with O'Brien et al. [20].

The authors found a strong correlation between the scaffold specific surface area and cell attachment indices. The cell attachment and viability are primarily influenced by scaffold specific surface area of pore sizes for MT3T3E1 cells.



**Fig. 6. (a) Load vs. displacement plot of different kinds of PCL-hydrogel specimens, (b) variation of interface fracture strength for various kinds of PCL-hydrogel specimens**

### 4. CONCLUSION

Cells were successfully grown in SA hydrogel with and without MgO nanoparticles. A significant viability difference was observed ( $p < 0.005$ ) between samples of hydrogel with and without MgO nanomedicine after 24 hours of cell culture. The interface tensile strength of PCL-SA hydrogel without MgO nanoparticles was 6 times greater than the hydrogel with MgO nanoparticles when cell-cultured for 2 weeks. Overall, PCL-SA hydrogel tissue constructs had the strongest tensile strength when only cells were present in the tissue constructs and the addition of nanoparticles decreased the adhesion between PCL and hydrogel.



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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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