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## Cross-Linked Gelatin Microcapsules for Drug Delivery in a Arthritic TMJ

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### 1. Introduction

A majority of individuals reporting temporomandibular joint (TMJ) disorders have joint damage, inflammation or arthritis (Manfredini, Chiappe & Bosco, 2006; Plesh, Sinisi, Crawford & Gansky, 2005). The joint will show loss of extra-cellular matrix components in the articular cartilage and subchondral bone resulting in destruction of cartilage and bone, such processes lead to inflammation and exacerbation of joint tissue catabolism (Tanaka, Detamore & Mercuri, 2008). Matrix metalloproteinases 1 and 9 (MMP-1 and MMP-9) are two major enzymes that contribute to tissue catabolism and have been observed in patients with TMJ disorders (Kanyama, Kuboki, Kojima, Fujisawa, Hattori, Takigawa & Yamashita, 2000; Srinivas, Sorsa, Tjaderhane, Niemi, Raustia, Pernu, Teronen & Salo, 2001; Yoshida, Takatsuka, Hatada, Nakamura, Tanaka, Ueki, Nakagawa, Okada, Yamamoto & Fukuda, 2006). Reversal of these disease processes and treatment of the joint to reduce pain are effective in the early stages of the disease, but treatment often fails to alleviate the severe, chronic pain caused by advanced joint degeneration (Gerwin, Hops & Lucke, 2006; Tanaka, Detamore & Mercuri, 2008).

Treatment of TMJ osteoarthritis with intra-articular injections of non-steroidal anti-inflammatory drugs (NSAIDs) and opiates into the superior joint space have shown efficacy (Bryant, Harrison, Hopper & Harris, 1999; Swift, Roszkowski, Alton & Hargreaves, 1998; Zuniga, Ibanez & Kozacko, 2007). Intra-articular administration versus systemic administration of NSAID or opiates would be advantageous for treatment of TMJ inflammation and pain because local administration avoids the ectopic effects seen with NSAIDs like rofecoxib (i.e., Vioxx) (Lin, Weisdorf, Solovey & Hebbel, 2000) or opiates. For example, nonselective NSAIDs can cause intestinal bleeding, whereas some selective cyclooxygenase-2 inhibitors have significant cardiovascular and renal safety risks (Davies & Jamali, 2004; Mukherjee, Nissen & Topol, 2001). Moreover, opioids frequently cause constipation, sedation, nausea, vomiting, and respiratory depression (Mercadante, 1999).

Intra-articular injection remains controversial in light of decades of mixed reports demonstrating intra-articular injections either accelerate or trigger destruction of tissues within the TMJ and the surrounding area (Bjornland, Rorvik, Haanaes & Teige, 1994; Sugisaki, Ikai & Tanabe, 1995; Westesson, Eriksson & Liedberg, 1986). Recent reports

demonstrate that the risks of intra-articular TMJ injection can be minimized by utilizing magnetic resonance imaging (MRI) for visualizing the needle during the injection procedure (Fritz, Thomas, Tzaribachev, Horger, Claussen, Lewin & Pereira, 2009; Hayakawa, Kober, Otonari-Yamamoto, Otonari, Wakoh & Sano, 2007). Current treatment protocols that utilize intra-articular drug delivery can require frequent injections, increasing the risk of infection and damage to the joint and, in the event MRI or some other form of imaging is utilized, dramatically increase the expense of the procedure. Alternatively, if drug delivery could be sustained after a single injection these risks from a multi-injection protocol could be minimized.

A variety of methods have been tested for sustained drug delivery in a joint space, including encapsulating or incorporating drugs into nano- or microparticles consisting of organic polymers (Deasy, 1994; Mountziaris, Kramer & Mikos, 2009). In this report we test a crosslinked fluid filled gelatin capsule for intra-articular injection. This capsule has a gelatin shell with a core of oil containing the drug ibuprofen or morphine. Other studies used uncrosslinked gelatin microcapsules, in contrast to our crosslinked capsules. The capsules were given orally but only released drug for 24-48 hours (Jizomoto, Kanaoka, Sugita & Hirano, 1993). Most intra-articular studies testing sustained release of NSAIDs utilized poly(lactide-co-glycolide) (PLGA) (Bozdog, Calis, Kas, Ercan, Peksoy & Hincal, 2001; Fernandez-Carballido, Herrero-Vanrell, Molina-Martinez & Pastoriza, 2004a; Fernandez-Carballido, Herrero-Vanrell, Molina-Martinez & Pastoriza, 2004b; Liggins, Cruz, Min, Liang, Hunter & Burt, 2004; Puebla, Pastoriza, Barcia & Fernandez-Carballido, 2005; Tuncay, Calis, Kas, Ercan, Peksoy & Hincal, 2000). PLGA has a rigid structure and may be irritating to the joint when injected intra-articularly, particularly when smaller than 20 microns (Liggins, Cruz, Min, Liang, Hunter & Burt, 2004). Importantly, in vivo studies injecting solid gelatin spheres have shown that they do not induce an inflammatory response in vivo suggesting gelatin in these studies should not induce an immune response (Brown, Leong, Huang, Dalal, Green, Haimes, Jimenez & Bathon, 1998).

Testing the rate of gelatin microcapsule degradation, as a result of MMP degradation, and the level of inflammation induced by the microcapsules in the TMJ are necessary. Also an important question to address was does sustained release improve pain reduction when administering NSAIDs or opiates to treat a TMJ disorder? To address these questions we used a rat model for TMJ arthritis.

In this model we inject the TMJ with the adjuvant complete Freund's adjuvant (CFA) and the rat's meal duration will lengthen in male and female rats as a result of this injection (Kerins, Carlson, Hinton, Grogan, Marr, Kramer, Spears & Bellinger, 2005; Kerins, Carlson, McIntosh & Bellinger, 2003; Kerins, Carlson, McIntosh & Bellinger, 2004; Kramer & Bellinger, 2009; Thut, Hermanstynne, Flake & Gold, 2007). Microcapsules containing vehicle or the NSAID ibuprofen or morphine were injected into the TMJ of rats that had no joint arthritis or had adjuvant (i.e., CFA) induce arthritis. Degradation of the microcapsules was monitored and the level of inflammatory cytokine IL-1 $\beta$  in the joint tissues was quantitated. In addition, the nociceptive response to the microcapsules loaded with vehicle or NSAID or morphine were measured in a non-arthritic and arthritic TMJ after intra-articular injection.

## 2. Materials and methods

### 2.1 Microcapsule production

To produce the loaded microcapsules canola oil was sonicated in water to induce formation of droplets 20-50 micrometers in size. Before sonication the canola oil was mixed with

2.5µl/ml of 1mM Alexa 488 dye (Invitrogen, Carlsbad CA) dissolved in toluene. Or a saturated solution of ibuprofen in canola oil was used containing ~15% (w/v) ibuprofen. A solution of Type A gelatin with a Bloom strength of 300 was added after sonication. The collagen gelatin was allowed to coat the oil droplets such that 80% of the microcapsule mass was canola oil and 20% was a gelatin shell by weight. Amino acids in the gelatin coated oil droplets were then cross-linked by addition of 15% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [EDC] (dry wt. of gelatin) to induce stable microcapsules. EDC is a water soluble carbodiimide usually obtained as the hydrochloride that can generally be used as a carboxyl activating agent for the coupling of primary amines to yield amide bonds between a free amine group (e.g., protein- or peptide-bound lysine) and the gamma-carboxamid group of protein- or peptide-bound glutamine. The cross-linking reaction was performed at room temperature for 12 hr. After crosslinking the microcapsules were washed 4 times with Tris Buffer pH 7.0, dried, sterilized with ethylene oxide and then hydrated with 0.9% saline. The morphine capsules were produced in a similar manner with some modifications. Morphine alkaloid crystals were ground to a small size (less than 50 microns) and suspended in canola oil. This suspension was emulsified in a solution of the gelatin at 60°C. A complexing agent (hexametaphosphate) was added to the aqueous phase and the pH was adjusted to approximately 4.75 with acetic acid to allow coacervation to occur. The microcapsules were crosslinked, lyophilized until dry and sterilized.

## 2.2 Detection of ibuprofen and morphine by HPLC

Known weights of beads were dissolved in acetonitrile and analyzed via high performance liquid chromatography (HPLC) as follows. A six point calibration curve was prepared by serial dilution and HPLC analysis. The stock solution of 1mg/mL was prepared in 1:1 deionized water (pH 2.5) and acetonitrile. Concentrations of 1, 5, 10, 25, 50 and 100 µg/mL were made in methanol. Samples of 20 µL were injected onto a C-18 column (Supelco Discovery C-18, 4.6 x 15 cm). The isocratic separation was carried out at 30°C using a mobile phase of 1:1 deionized water (pH 2.5) and acetonitrile. The flow rate was 2.0 ml/min with a run time of 10 minutes. Absorbance at 214 nm was taken using a Waters 486 UV detector. The absorbance of the sample was compared to a six-point standard curve from which concentration and percent loading in capsules was determined.

## 2.3 In vitro assay for microcapsule degradation

In 24 well tissue culture plates approximately 80,000 microcapsules were placed in each well with either 0.9% saline, 15 µg/ml CFA (paraffin oil/15µg *Mycobacterium tuberculosis*) mixed in water, or a 50:50 mixture of 0.9% saline and 15 µg/ml CFA. A homogenate of the water and oil was created by passing the solution through a 20 gauge needle 5 times. The plates were placed in a 5% CO<sub>2</sub> chamber at 37°C. The microcapsules were counted in triplicate in a hemacytometer chamber 0, 5 and 10 days following addition to the different solutions.

## 2.4 TMJ Injections

The Baylor College of Dentistry Institutional Animal Care and Use Committee approved the experimental protocol. Male (250 grams) Sprague-Dawley rats from Harlan Industries, Houston, TX were kept on a 12:12 light/dark cycle with lights on at 08:00 hours. The animals were housed individually in our computerized feeding modules and given food and water ad libitum. They were acclimated to the surroundings for two days before

receiving injections. Prior to the TMJ injections, the rats were removed from their cages and anesthetized with a 5% gas flow of isoflurane. Following the TMJ injections and removal from anesthesia, the rats began freely moving within two minutes.

#### **2.4.1 Bead degradation study**

Saline or 15  $\mu\text{g}$  of CFA in 15  $\mu\text{l}$  was injected into the upper joint space of the TMJ followed by a second injection 24 hours later of 30  $\mu\text{l}$  of dye loaded microcapsules (90 mg microcapsules [dry weight]/1 ml Tris buffer or 0.9% saline) into the same location. The animals were sacrificed 5 hours (day 0), 5 and 10 days after injection of microcapsules and TMJ was removed en bloc, fixed in paraformaldehyde, sectioned and stained as described below.

#### **2.4.2 Microcapsules affect nociceptive response study**

Rats were injected with 30  $\mu\text{l}$  0.9% saline or 15  $\mu\text{g}$  CFA and then 24 hours later the rats were injected with either saline or 30  $\mu\text{l}$  of the microcapsule slurry (20% w/v) in 0.9% saline. Before and after injection of the microcapsules the meal duration was recorded.

#### **2.4.3 MMP effect on degradation study**

Rats were injected with 30  $\mu\text{l}$  saline or 15  $\mu\text{g}$  CFA or saline with an MMP inhibitor or CFA with an MMP inhibitor added and then 24 hours later these groups of rats were injected with 30  $\mu\text{l}$  microcapsules or microcapsules plus the MMP inhibitor. The MMP inhibitor was a 1  $\mu\text{M}$  solution of the MMP-2,9 inhibitor IV (EMD Biosciences, catalogue # 444274). In the first experiment the rats were sacrificed 0, 5 and 10 days following microcapsule injection, the TMJ was removed, sectioned and the microcapsules counted. In the second experiment the rats nociceptive response was assayed in the pellet feeders, as described below.

#### **2.4.4 Drug loaded microcapsules study**

Rats were injected into the upper joint space of the TMJ with a 30  $\mu\text{l}$  slurry of microcapsules containing 15% ibuprofen or 1% morphine in oil or a slurry of microcapsules containing just oil. After twenty-four hours a portion of these animals were subdivided further; one group received bilateral injections of 0.9% saline (30  $\mu\text{l}$ ) and the other group received an injection of CFA into the upper joint space. Before and after injection the nociceptive response (i.e., meal duration) was assayed by placing the rats in pellet feeders. Also, on day 7 post-injection the TMJ tissue of a portion of these rats was harvested for quantitation of the cytokine IL-1 $\beta$ .

### **2.5 Nociceptive assay**

The rats were housed individually in sound-attenuated chambers equipped with photobeam computer-activated pellet feeders (Med Assoc. Inc., East Fairfield, VT). The rats were given 45 mg rodent chow pellets (Product No. FO 165, Bioserv, Frenchtown, NJ). When a rat removes a pellet from the feeder trough, a photobeam placed at the bottom of the trough is no longer blocked, signaling the computer to drop another pellet, record the date and time, and keep a running tally of the total daily food consumption. The computer record of pellets dropped over time establishes the meal duration, which is a continuous non-invasive biological marker of TMJ nociception (surface and deep) in undisturbed male



and female rats (Kerins, Carlson, Hinton, Grogan, Marr, Kramer, Spears & Bellinger, 2005; Kerins, Carlson, McIntosh & Bellinger, 2003).

### 2.6 Animals and tissue preparation

Rats were sacrificed within 20 sec to minimize stress. For the cytokine study the TMJ anterior, disc, retrodiscal and synovium were dissected from one side by performing a superficial, horizontal skin incision parallel and just inferior to the zygomatic arch. The masseter and temporalis muscles were cut away from the arch and the ramus of the mandible so that the arch could be removed with rongeurs. The neck of the condyle was grasped with hemostats and the condylar neck fractured to allow removal of any remaining musculature and access to the anterior, disc, retrodiscal and synovial tissues. Dissected TMJ tissue was excised from the posterior neck of the condyle, and all anterior tissue, articular disc, retrodiscal tissue and synovium were removed, placed in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . To count the number of microcapsules in the TMJ a 0.5 cm cube of TMJ tissue centered on the condyle from the other side was removed and placed in 4% paraformaldehyde at  $4^{\circ}\text{C}$  overnight. The tissue was then decalcified in 0.5 M EDTA in a Pelco microwave (Ted Pella Inc., Redding CA) for 2 weeks, frozen and 20  $\mu\text{m}$  sections were cut and mounted on Superfrost glass slides (Fisher Scientific, Pittsburgh, PA). Every 5<sup>th</sup> section was stained with hematoxylin and eosin for counting and a small number of the slides were mounted with fluormount and DAPI. Microcapsules were counted on every 5<sup>th</sup> section to obtain a representative sample through the entire TMJ region (West & Slomanka, 2001). The fluorescent signal was imaged using MetaMorph Imaging System software (Molecular Devices Corporation, Sunnyvale, CA), a Photometrics CoolSnap K4 CCD camera (Roper Scientific, Inc, Duluth, GA) and a fluorescent microscope using a DAPI filter with excitation between 395-410 nm and an emission between 450-470 nm, as well as, a FITC filter with excitation between 490-505 nm and an emission between 515-545 nm.

### 2.7 Cytokine assay

The TMJ tissue was homogenized in buffer (75 mM potassium acetate pH 7.4, 300 mM NaCl, 10 mM EDTA, 0.25% Triton X-100, protease inhibitors). It has previously been shown that TMJ-injected CFA can increase IL-1 $\beta$  (Kerins, Carlson, McIntosh & Bellinger, 2003). Therefore, the cytokines IL-1 $\beta$  were quantitated from this homogenate using ELISA kits according to the manufacturer's directions (R&D Systems, Minneapolis, MN).

## 3. Statistics

The data were analyzed using a two-way analysis of variance with repeated measurement. Independent variables were treatment and time. The dependent variable was either the bead count or meal duration. The data found to be significant were further analyzed by Bonferonni pos-hoc test.

## 4. Results

Images of the microcapsules in solution show that the capsules average size was 30  $\mu\text{m}$  (Fig. 1A) and that the inside of the microcapsule is loaded with the dye/mineral oil mixture (Fig. 1B). About 80% of the mass of these microcapsules is the fluid mineral oil and about 20% of the mass consists of the gelatin shell.

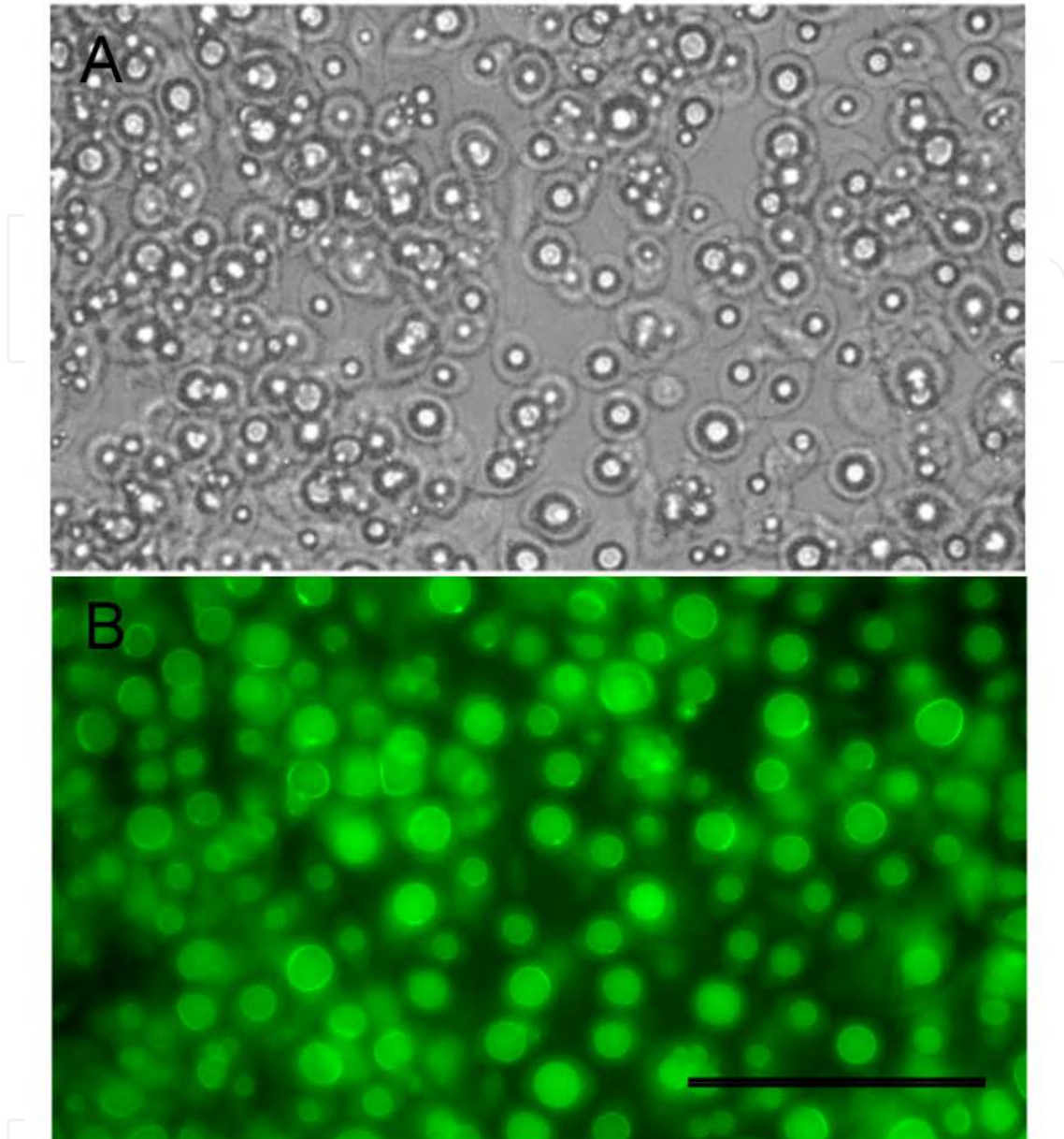


Fig. 1. Gelatin microcapsules containing canola oil mixed with Alexa 488 dye range in size between 15-30 micrometers. Microcapsules are loaded with canola oil (80% by weight) mixed with 1mM Alexa 488 dye. A) Bright field image of the microcapsules in solution. B) Fluorescent image of the same microcapsules before injection into the TMJ. Bar equals 100  $\mu$ M.

To initially assess the degradation characteristics of the gelatin shell the microcapsules were placed in 24 well tissue culture plates containing saline and CFA solutions. Over a period of 10 days the beads did significantly degrade in a mixture of isotonic saline and 15  $\mu$ g of CFA (Fig. 2).

Injection of dye loaded microcapsules (30  $\mu$ l) into the upper joint space, a region between the articular fossa and condyle (Fig. 3A) produced an area of beads 2-3 mm in diameter (dotted line, Fig. 3B) in the upper joint space of the rat TMJ. Cells (e.g. neutrophils) were adjacent to the microcapsules 5 hours after injection (blue nuclei, Fig. 3C-E). In the TMJ

the microcapsules appeared to degrade as a result of inducing an arthritic response (i.e., CFA injection), compare Fig. 4A to Fig. 4B and compare Fig. 4C to Fig. 4D. Time also reduced the number of microcapsules in the joint, compare 5 day and 10 day saline injected rats (compare Fig. 4A to Fig. 4C) and 5 day and 10 day CFA injected rats (compare Fig. 4B to Fig. 4D). A significant decrease in the number of intact beads was observed in the inflamed TMJ when comparing the 5 day time point to the 10 day time point (Fig. 4E).

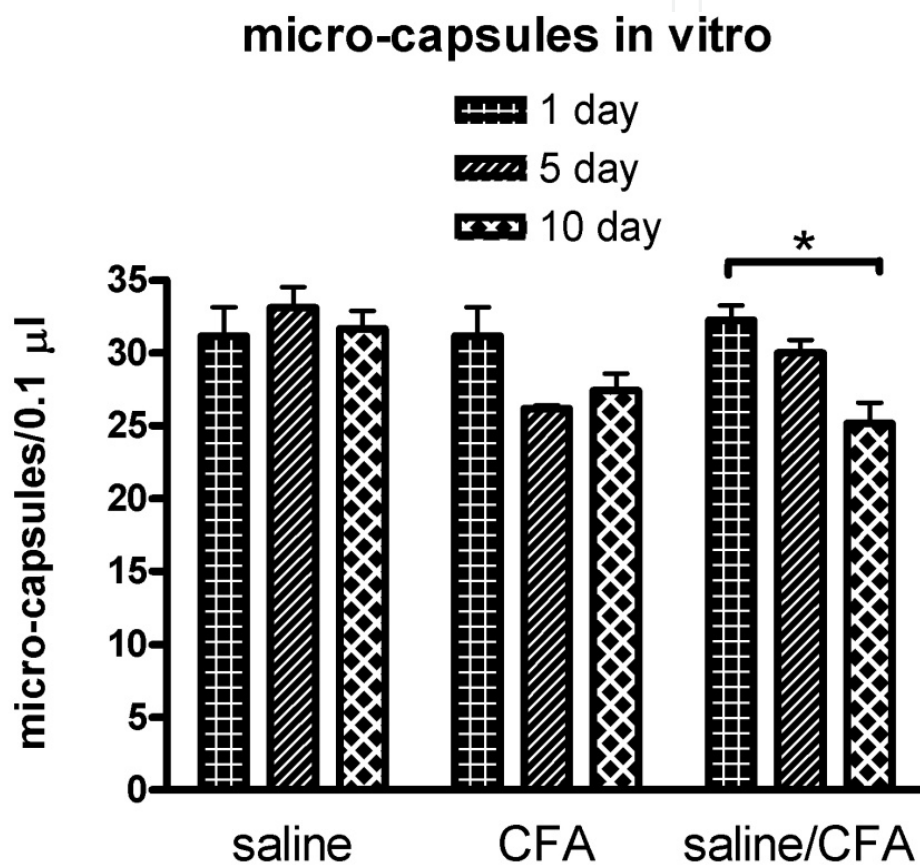


Fig. 2. Microcapsule breakdown in vitro. Microcapsules (approximately 80,000) were placed in 200  $\mu$ l of three different solutions at a temperature of 37°C and the degradation of the microcapsules was monitored by counting the number of intact microcapsules remaining after 1, 5 and 10 days. The solutions the microcapsules were placed in consisted of either 1) 0.9% saline or 2) 15  $\mu$ g/ml of CFA/water homogenate or 3) a 50%/50% homogenate of 0.9% saline and 15  $\mu$ g/ml CFA. At each time point a 10  $\mu$ l aliquote was removed and the number of microcapsules was counted in triplicate in a hemacytometer chamber. Values are the mean  $\pm$  SEM.



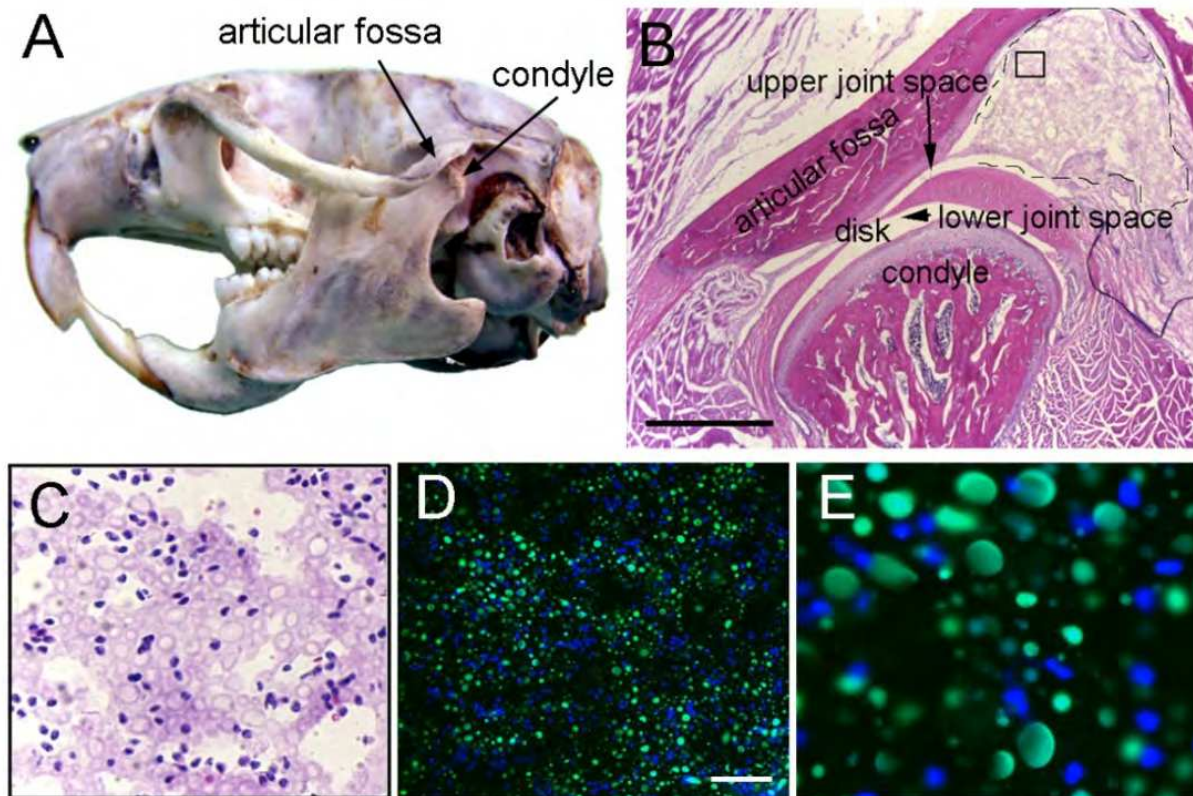


Fig. 3. Injection of gelatin microcapsules containing canola oil with Alexa 488 dye were injected into the upper joint space of a rat temporomandibular joint (TMJ). A) Sagittal view of a rat skull, arrows point to the TMJ condyle and articular fossa. In this experiment the TMJ upper joint space was injected with 30  $\mu$ l of dye loaded microcapsules (90 mg microcapsules [dry weight] in 1 ml of Tris buffer or 0.9% saline and then the rats were sacrificed 5 hours later. B) Saggittal section of a rat TMJ 5 hours after injection of microcapsules into the upper joint space (arrow) stained with hemotoxylin and eosin. Dotted line in the upper joint space outlines the region containing the injected microcapsules. C) High magnification image of boxed region in panel B. D) Fluorescent image of Alexa 488 loaded microcapsules 5 hours after injection into the TMJ. Green is the Alexa 488 loaded microcapsules and blue is the DAPI stained cell nuclei. E) High magnification of image from panel D. Bar in panel B equals 1 mm and bar in panel D equals 100  $\mu$ m.

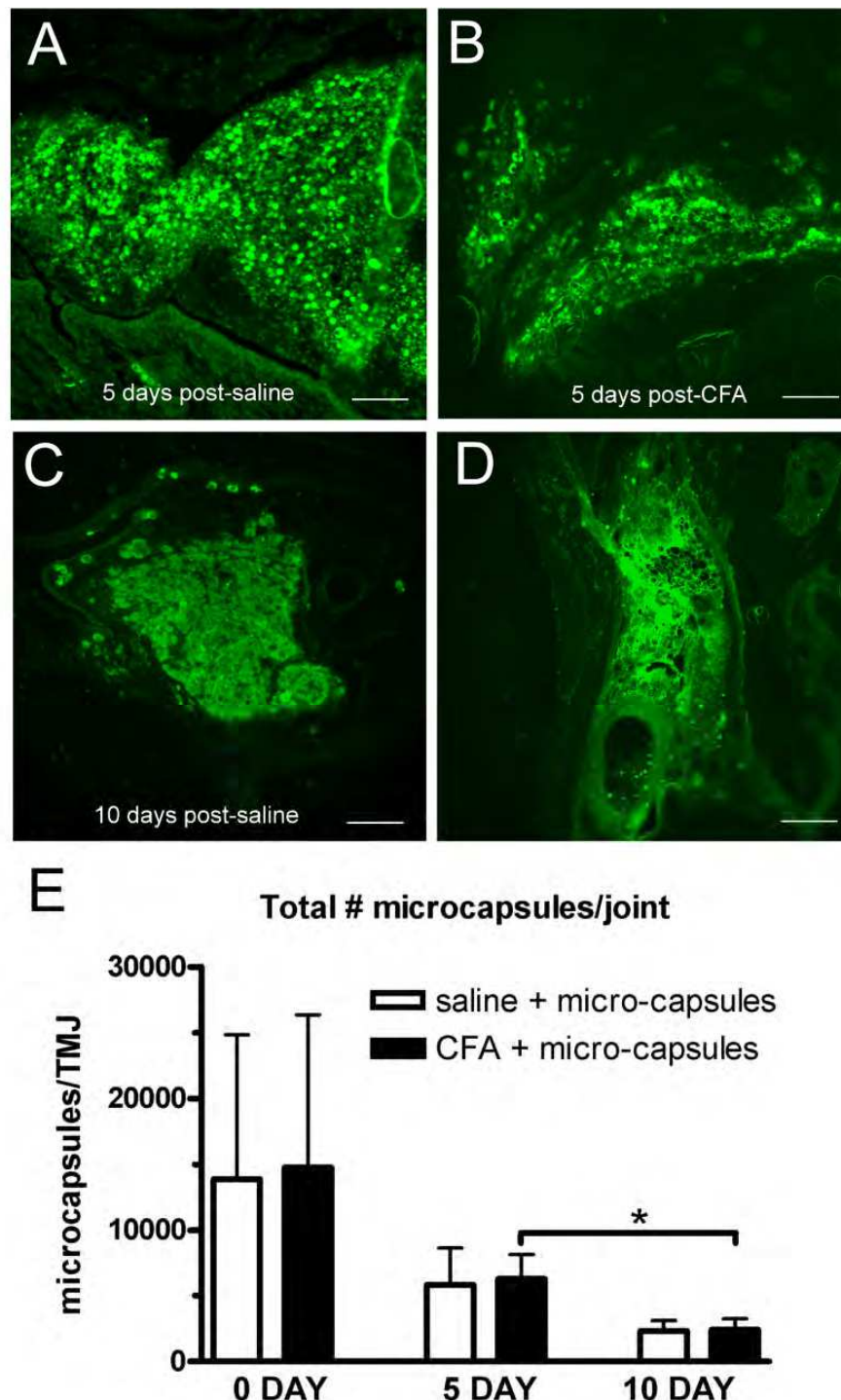


Fig. 4. Microcapsule breakdown in a non-arthritic and arthritic TMJ. Saline or 15  $\mu\text{g}$  of CFA in 15  $\mu\text{l}$  was injected into the TMJ followed by a second injection 24 hours later of 30  $\mu\text{l}$  of dye loaded microcapsules. Microcapsules in the upper joint space/synovial tissue were imaged in sagittal sections of the TMJ tissue 5 and 10 days after injection of microcapsules. Images were captured 5 days after injecting the TMJ with microcapsules and saline (A) or CFA (B). Microcapsules in the upper joint space/synovial tissue was imaged 10 days after injection of microcapsules in a joint previously injected with saline (C) or CFA (D). The histogram below indicates the number of microcapsules counted per joint in rats sacrificed 0, 5 and 10 days after injection. There were 5 animals per group. Asterisk indicates  $P < 0.05$ .

We next asked the question, would injection of microcapsules into the TMJ induce a pain response in the rat with a non-arthritic or an arthritic joint. To test the response to microcapsule injection we measured a behavioral correlate of nociception (meal duration) before and after injecting microcapsules into the TMJ of a rat previously injected with saline (non-arthritis) or CFA (arthritic). Microcapsule injection did not significantly increase the pain response in either the non-arthritic or arthritic joint (Fig. 5).

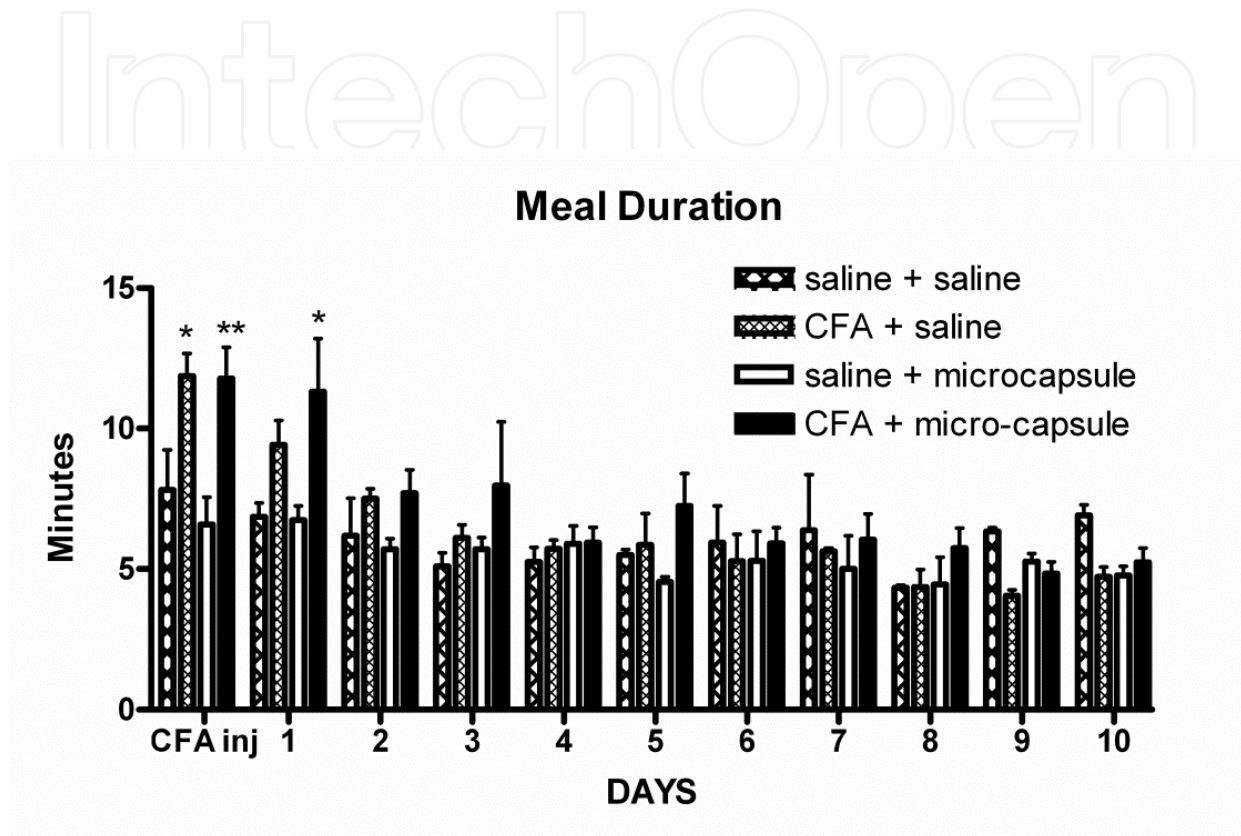


Fig. 5. Nociceptive response after injecting microcapsules into a non-arthritic and arthritic TMJ. Rats were injected with 30  $\mu$ l 0.9% saline or 15  $\mu$ g CFA (CFA inj) and then 24 hours later the rats were injected with either saline or 30  $\mu$ l microcapsules in 0.9% saline. After injection of the microcapsules the meal duration was recorded for 10 days (1 through 10). There were 8 animals per group.  $*$ = $P$ <0.05,  $**$ = $P$ <0.01 when comparing the CFA + saline group to the saline + saline group and when comparing the CFA + microcapsule group to the saline + microcapsule group.

To test that the breakdown of beads in vivo may be due the presence of MMP-2 and MMP-9 we injected the microcapsules with an MMP-2/9 inhibitor. The number of microcapsules decreased over time in vivo but addition of a MMP-2/9 inhibitor had no significant effect on microcapsules degradation after injection into a rat TMJ (Fig. 6). The inhibition of the MMP-2/9 also did not affect the nociceptive response after injecting the TMJ with microcapsules (Fig. 7).



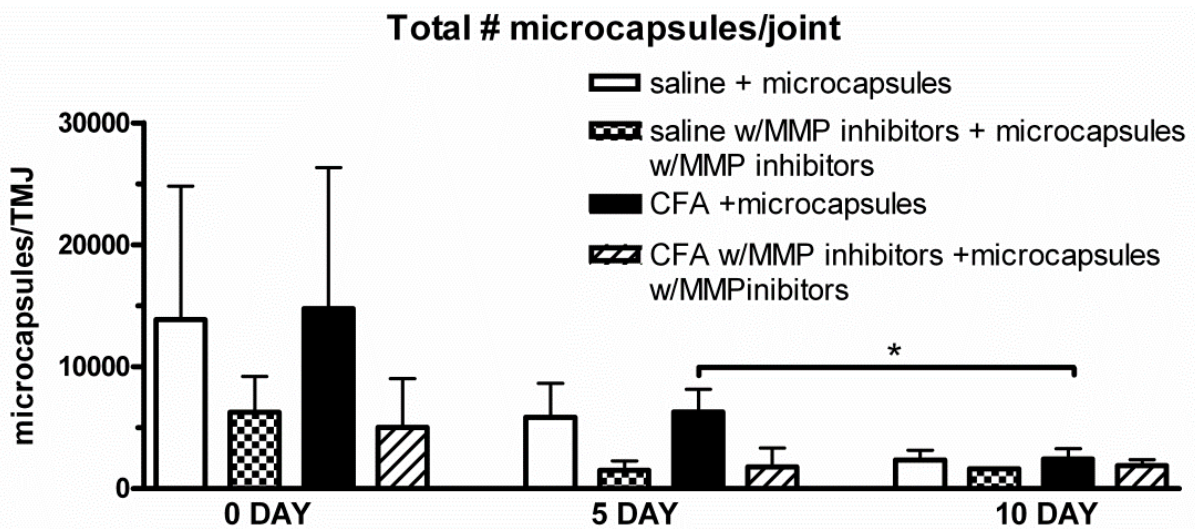


Fig. 6. Effect of metalloprotease inhibitor 2 and 9 on microcapsule breakdown in vivo Rats were injected with saline or CFA or saline with an MMP inhibitor or CFA with an MMP inhibitor added and then 24 hours later these groups of rats were injected with 30  $\mu$ l microcapsules or microcapsules plus MMP inhibitor. All injections were into the superior joint space of the TMJ. The inhibitor was a 1  $\mu$ M solution of the MMP-2/9 inhibitor IV. The histogram shows the total number of beads per joint after 0, 5 and 10 days after bead injection. There were 3-5 animals per group.  $^* = P < 0.05$ .

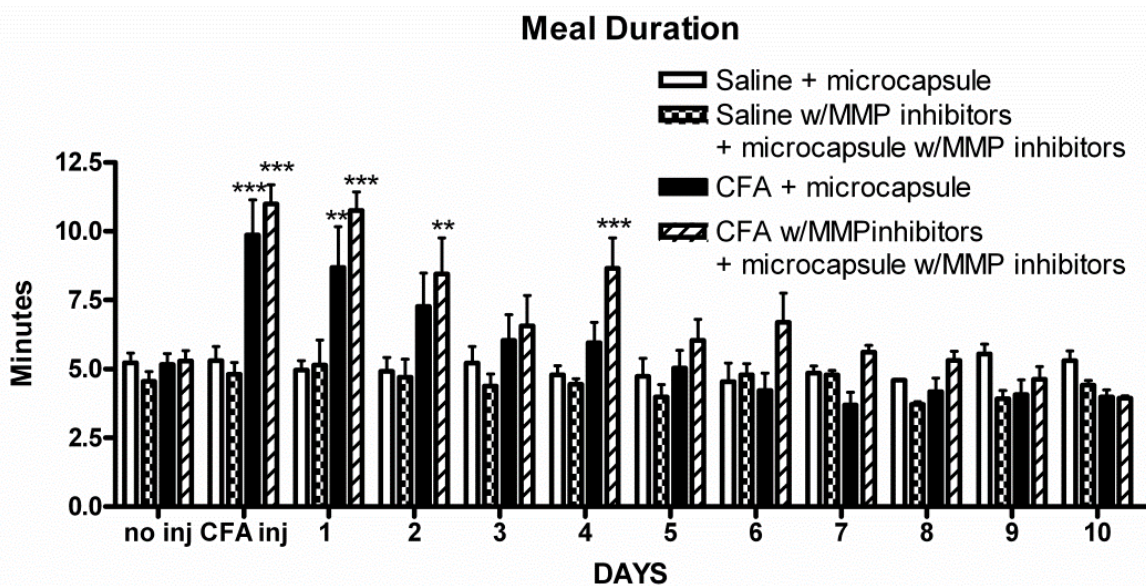


Fig. 7. Nociceptive response after injecting microcapsules along with metalloprotease inhibitors Rats were injected into the upper joint space of the TMJ with 30  $\mu$ l saline or 15  $\mu$ g CFA (CFA inj), some in combination with a MMP-2/9 inhibitor. One day after this injection a second 30  $\mu$ l injection of microcapsules or microcapsules containing MMP-2/9 inhibitor was performed. Before and after injection the meal duration was recorded. No inj= before animal were injected. There were 8 animals per group.  $^* = P < 0.05$ ,  $^{**} = P < 0.01$ ,  $^{***} = P < 0.001$  when comparing CFA injected rats to the saline injected rats.



Ibuprofen drug loaded microcapsules did not significantly affect the nociceptive response (Fig. 8A) or the immune response (Fig. 8B) of CFA injected rats. Morphine loaded microcapsules did significantly decrease the nociceptive response ( $p < 0.05$ , days 4 and 5) and post-hoc testing showed a significant decrease on the fourth day post-CFA injection (Fig. 8A).

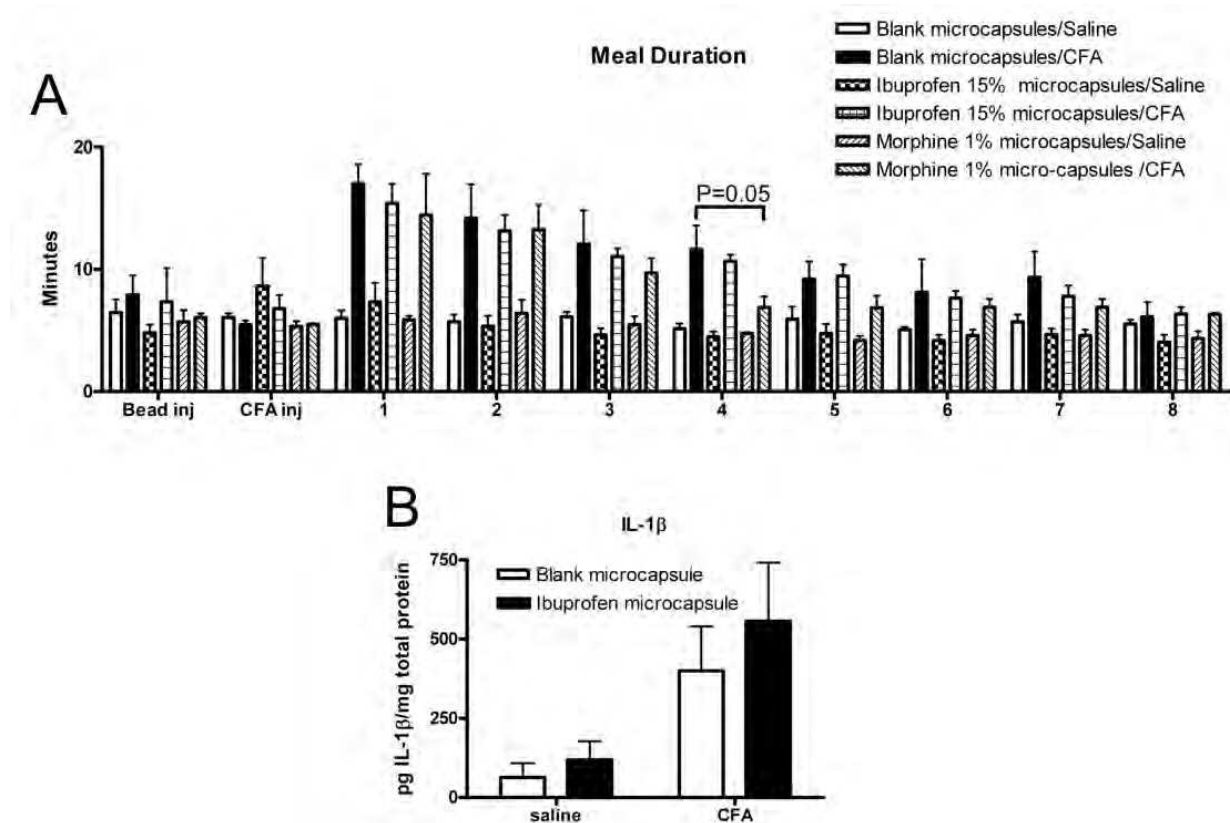


Fig. 8. Nociceptive and inflammatory response after injecting microcapsules containing ibuprofen or morphine. Rats TMJs were injected with a 30  $\mu$ l solution of microcapsules (bead inj). The microcapsules contained oil (Blank microcapsules) or oil with 15% ibuprofen (Ibuprofen microcapsules) or oil containing 1% morphine (Morphine microcapsules). 24 hours after microcapsule injection the rats were subdivided further for TMJ injection of either saline or 15  $\mu$ g CFA (CFA inj). A) Before and 8 days after injection of the saline/CFA the daily meal duration was recorded. There were 8 animals in each of the four treatment groups. B) The amount of IL-1 $\beta$  in the TMJ retrodiscal, synovial and disc tissue 7 days after injection with microcapsules. There were 3-4 animals in each of the four treatment groups for the cytokine analysis.

## 5. Discussion

Gelatin microcapsules have the capability for sustained drug release after intra-articular injection (Inoue, Takahashi, Arai, Tonomura, Sakao, Saito, Fujioka, Fujiwara, Tabata & Kubo, 2006; Lu, Zhang, Sun & Zhong, 2007; Saito, Takahashi, Arai, Inoue, Sakao, Tonomura, Honjo, Nakagawa, Inoue, Tabata & Kubo, 2009). Sustained drug delivery into a diseased TMJ has the potential to improve the treatment of TMJ pain and inflammation. In this report a rat model of TMJ inflammatory arthritis was used and we tested the effect of intra-

articularly injected gelatin microcapsules on the nociceptive response as well as on the inflammatory response. Loading the gelatin microcapsules with the ibuprofen or morphine was accomplished because we hypothesized that intra-articular injection of drug loaded beads would reduce the nociceptive response. We determined that gelatin microcapsules do not increase IL-1 $\beta$  levels in the disc and synovial tissues after injection into the TMJ. Nor do the capsules increase the nociceptive response in an arthritic joint. Importantly, injection of morphine but not ibuprofen loaded microcapsules decreased the nociceptive response of a rat with an arthritic TMJ. Because injection of microcapsules loaded with morphine decreased the nociceptive response of a rat with inflammatory arthritis we expect that the microcapsules will ameliorate the pain response in patients with TMJ disease.

### 5.1 TMJ intra-articular model for testing microcapsules

The premise of our animal (i.e., meal duration) model is that the CFA induced TMJ pain would affect the rat, such that, when a hungry animal initiated a meal the animal would eat slowly due to the TMJ pain associated with the movement of the mandible during the chewing process. This is exactly what we observed following bilateral TMJ CFA injections in males and females, i.e., the rats had longer meal durations (Harper, Kerins, Talwar, Spears, Hutchins, Carlson, McIntosh & Bellinger, 2000; Kerins, Carlson, Hinton, Grogan, Marr, Kramer, Spears & Bellinger, 2005; Kerins, Carlson, McIntosh & Bellinger, 2003). Support that meal duration is a measure for orofacial pain is that when ibuprofen is administered directly into a inflamed, CFA-injected TMJ meal duration was normal in both male and female rats (Kerins, Carlson, McIntosh & Bellinger, 2003). Our selection of CFA was made because it produces a persistent arthritic pain response that last for over two weeks (Hill, Bellinger, Spears, Hutchins, Kerins & Kramer, 2007; Ren, 1999). Thus, of all the agents, CFA was best when trying to establish long duration pain. Injection of CFA into the TMJ significantly lengthened meal duration in rats, while the same amount of CFA in the knee did not affect meal duration (Kerins, Carlson, Hinton, Grogan, Marr, Kramer, Spears & Bellinger, 2005) indicating meal duration is a specific measure of orofacial pain. Interestingly IL-1 $\beta$  remained significantly elevated in the TMJ of the ibuprofen treated animals injected with CFA (Kerins, Carlson, McIntosh & Bellinger, 2003), suggesting that some inflammation from the CFA injection remained. In another study, cyclooxygenase-II (COX-2) inhibitors normalized meal duration in rats after CFA injection (Kerins, Carlson, McIntosh & Bellinger, 2004). In this study the COX-2 inhibitor also attenuated the inflammation, i.e., TMJ tissue IL-1 $\beta$  normalized (Kerins, Carlson, McIntosh & Bellinger, 2004). In still another study, rats were given capsaicin or vehicle at 2 and 10 days of age; capsaicin permanently destroyed afferent nociceptive fibers in these animals (Bellinger, Spears, King, Dahm, Hutchins, Kerins & Kramer, 2007). When these male rats reached adulthood saline or CFA was injected into the TMJ and their meal duration was measured. Capsaicin treatment alone had no effect on meal duration, because saline injected, non-capsaicin treated rats had the same meal duration as saline injected, capsaicin treated rats. Non-capsaicin treated rats injected with CFA had longer meal durations than rats that were pre-treated with capsaicin, which demonstrated meal duration after CFA injection was normalized due to a capsaicin-induced loss of afferent nociceptive neuronal fibers (Bellinger, Spears, King, Dahm, Hutchins, Kerins & Kramer, 2007). The lack of change in meal duration in these capsaicin treated male rats occurred despite CFA inducing greater TMJ swelling, which demonstrated that the physical and mechanical changes in the inflamed TMJ synovial joint did not affect meal duration measurements. Another rationale for suggesting that meal duration is a measure of

nociception stems from the finding that eating is impaired in patients with TMD (Haketa, Kino, Sugisaki, Amemori, Ishikawa, Shibuya, Sato & Yoshida, 2006) and from a clinical study of juvenile rheumatoid arthritic children (Harper, Brown, Triplett, Villasenor & Gatchel, 2000) that examined chewing performance as an objective measure of masticatory function. It showed that the juvenile rheumatoid arthritic children with TMD symptoms changed their chewing habits presumably to “guard” against pain. Most recently, meal duration in the rat was shown to be increased over the course of a week following pulp exposures demonstrating meal duration can also be used as a measure of tooth nociception (Bellinger, He & Kramer, 2010).

### **5.2 Sustained drug release by gelatin is safe and effective**

Gelatin microcapsules are one type of sustained drug release mechanism that incorporates a shell of gelatin around a core of oil containing drug. One advantage of gelatin is it has been shown to be a safe means for sustained drug delivery because *in vivo* there is no inflammatory response (Brown, Leong, Huang, Dalal, Green, Haimes, Jimenez & Bathon, 1998; Tuncay, Calis, Kas, Ercan, Peksoy & Hincal, 2000) consistent with our result in the TMJ of a rat that showed injection of microcapsules did not increase the nociceptive response nor increased IL-1 $\beta$  levels in the TMJ. Studies using a shell of gelatin encapsulating oil containing drug demonstrated that uncrosslinked gelatin was effective when given orally (Jizomoto, Kanaoka, Sugita & Hirano, 1993). A previous pharmacokinetic study injecting gelatin microspheres containing NSAID show that the intra-articular concentration of NSAID was 8-fold higher 8 hours after injection versus the concentration of NSAID in a joint injected with a non-encapsulated solution of NSAID (Lu, Zhang, Sun & Zhong, 2007). Another recent study in a rabbit model indicated that intra-articular delivery of basic fibroblast growth factor or platelets via gelatin microspheres improved knee joint swelling, proteoglycan expression, and histological appearance of arthritic knee tissue (Inoue, Takahashi, Arai, Tonomura, Sakao, Saito, Fujioka, Fujiwara, Tabata & Kubo, 2006; Saito, Takahashi, Arai, Inoue, Sakao, Tonomura, Honjo, Nakagawa, Inoue, Tabata & Kubo, 2009). Our work is consistent with these results demonstrating microcapsule drug release can ameliorate arthritic TMJ pain. Future work would focus on encapsulating alternative drugs shown to be effective in relieving TMJ pain, such as mepivacaine (Zuniga, Ibanez & Kozacko, 2007).

### **5.3 Microcapsule size and thickness for *in vivo* injection**

Thickness of the microcapsule wall can be varied and the timing of drug release would be based on the capsule cell wall thickness. What is important to keep in mind is that the microcapsules for this application need to be injectable and thus the diameter should not exceed the inner bore of the needle (e.g. 165  $\mu$ M for a 29 gauge needle). As the wall of the bead becomes thicker the amount of loaded drug decreases thus, you inject less drug when using beads with a thicker wall diameter. Thus, a balance between the amount of time you want before drug release and the total amount of drug administered must be obtained. The diameter of the sphere is also important, with spheres greater than 26  $\mu$ M remaining in the interstitial fluid of the tissue because they are not phagocytosed by cells (e.g., macrophages) (Horisawa, Kubota, Tuboi, Sato, Yamamoto, Takeuchi & Kawashima, 2002). The optimal size range for intra-articular drug delivery systems is application specific (Horisawa, Hirota, Kawazoe, Yamada, Yamamoto, Takeuchi & Kawashima, 2002; Horisawa, Kubota, Tuboi, Sato, Yamamoto, Takeuchi & Kawashima, 2002; Liggins, Cruz, Min, Liang, Hunter & Burt,

2004) but in this intra-articular paradigm we did not want intracellular drug release thus the bead diameter was approximately 30  $\mu$ M.

In conclusion drug loaded gelatin microcapsules reduce the nociceptive response of an arthritic TMJ. These microcapsules are expected to be useful not only for the treatment of pain but also to modify the joint environment prior to implantation by delivering pro-regenerative signals in a spatially and temporally controlled fashion. The limitations of current therapeutic strategies for TMJ disorders have led to increased interest in tissue engineering strategies, which combine cells, bioactive factors, and implantable scaffolds to trigger joint regeneration (Detamore & Athanasiou, 2003). In addition to the added benefit of sustained drug release local administration of drug has the added benefit of avoiding systemic drug release which can result in ectopic effects.

## 6. References

- [1] Bellinger, L. L., He, L., and Kramer, P. R., 2010, Meal duration; a measure of tooth and temporomandibular joint nociception.: American Association of Dental Research CD-ROM, p. 1267.5/AA31, Washington, DC.
- [2] Bellinger, L. L. Spears, R. King, C. M. Dahm, F. Hutchins, B. Kerins, C. A. & Kramer, P. R. (2007), Capsaicin sensitive neurons role in the inflamed TMJ acute nociceptive response of female and male rats: *Physiology and Behavior*, Vol. 90, pp. 782-789.
- [3] Bjornland, T. Rorvik, M. Haanaes, H. R. & Teige, J. (1994), Degenerative changes in the temporomandibular joint after diagnostic arthroscopy. An experimental study in goats.
- [4] Bozdag, S. Calis, S. Kas, H. S. Ercan, M. T. Peksoy, I. & Hincal, A. A. (2001), In vitro evaluation and intra-articular administration of biodegradable microspheres containing naproxen sodium: *J Microencapsul.*, Vol. 18, pp. 443-456.
- [5] Brown, K. E. Leong, K. Huang, C. H. Dalal, R. Green, G. D. Haimes, H. B. Jimenez, P. A. & Bathon, J. (1998), Gelatin/chondroitin 6-sulfate microspheres for the delivery of therapeutic proteins to the joint: *Arthritis Rheum*, Vol. 41, pp. 2185-2195.
- [6] Bryant, C. J. Harrison, S. D. Hopper, C. & Harris, M. (1999), Use of intra-articular morphine for postoperative analgesia following TMJ arthroscopy: *British Journal of Oral and Maxillofacial Surgery*, Vol. 37, pp. 391-396.
- [7] Davies, N. M. & Jamali, F. (2004), COX-2 selective inhibitors cardiac toxicity: getting to the heart of the matter: *J Pharm.Pharm.Sci*, Vol. 7, pp. 332-336.
- [8] Deasy, P. B. (1994), Evaluation of drug-containing microcapsules: *J Microencapsul.*, Vol. 11, pp. 487-505.
- [9] Detamore, M. S. & Athanasiou, K. A. (2003), Motivation, characterization, and strategy for tissue engineering the temporomandibular joint disc: *Tissue Eng*, Vol. 9, pp. 1065-1087.
- [10] Fernandez-Carballido, A. Herrero-Vanrell, R. Molina-Martinez, I. T. & Pastoriza, P. (2004a), Biodegradable ibuprofen-loaded PLGA microspheres for intraarticular administration. Effect of Labrafil addition on release in vitro: *Int.J Pharm.*, Vol. 279, pp. 33-41.
- [11] Fernandez-Carballido, A. Herrero-Vanrell, R. Molina-Martinez, I. T. & Pastoriza, P. (2004b), Sterilized ibuprofen-loaded poly(D,L-lactide-co-glycolide) microspheres for intra-articular administration: effect of gamma-irradiation and storage: *J Microencapsul.*, Vol. 21, pp. 653-665.

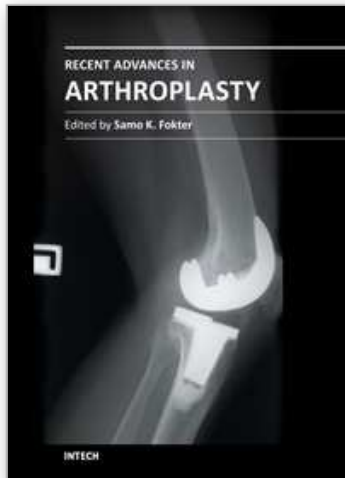


- [12] Fritz, J. Thomas, C. Tzaribachev, N. Horger, M. S. Claussen, C. D. Lewin, J. S. & Pereira, P. L. (2009), MRI-guided injection procedures of the temporomandibular joints in children and adults: technique, accuracy, and safety.
- [13] Gerwin, N. Hops, C. & Lucke, A. (2006), Intraarticular drug delivery in osteoarthritis: *Adv. Drug Deliv. Rev.*, Vol. 58, pp. 226-242.
- [14] Haketa, T. Kino, K. Sugisaki, M. Amemori, Y. Ishikawa, T. Shibuya, T. Sato, F. & Yoshida, N. (2006), Difficulty of food intake in patients with temporomandibular disorders: *Int J Prosthodont.*, Vol. 19, pp. 266-270.
- [15] Harper, R. P. Brown, C. M. Triplett, M. M. Villasenor, A. & Gatchel, R. J. (2000), Masticatory function in patients with juvenile rheumatoid arthritis: *Pediatr. Dent.*, Vol. 22, pp. 200-206.
- [16] Harper, R. P. Kerins, C. A. Talwar, R. Spears, R. Hutchins, B. Carlson, D. S. McIntosh, J. E. & Bellinger, L. L. (2000), Meal pattern analysis in response to temporomandibular joint inflammation in the rat: *J. Dent. Res.*, Vol. 79, pp. 1704-1711.
- [17] Hayakawa, Y. Kober, C. Otonari-Yamamoto, M. Otonari, T. Wakoh, M. & Sano, T. (2007), An approach for three-dimensional visualization using high-resolution MRI of the temporomandibular joint: *Dentomaxillofac. Radiol.*, Vol. 36, pp. 341-347.
- [18] Hill, G., Bellinger, L. L., Spears, R., Hutchins, B., Kerins, C., and Kramer, P. R., 2007, Operant behavioral model for studying persistent orofacial nociception: The Winter Conference on Animal Learning and Behavior, Winter Park, CO.
- [19] Horisawa, E. Hirota, T. Kawazoe, S. Yamada, J. Yamamoto, H. Takeuchi, H. & Kawashima, Y. (2002), Prolonged anti-inflammatory action of DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate for an intra-articular delivery system in antigen-induced arthritic rabbit: *Pharm. Res.*, Vol. 19, pp. 403-410.
- [20] Horisawa, E. Kubota, K. Tuboi, I. Sato, K. Yamamoto, H. Takeuchi, H. & Kawashima, Y. (2002), Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium: *Pharm. Res.*, Vol. 19, pp. 132-139.
- [21] Inoue, A. Takahashi, K. A. Arai, Y. Tonomura, H. Sakao, K. Saito, M. Fujioka, M. Fujiwara, H. Tabata, Y. & Kubo, T. (2006), The therapeutic effects of basic fibroblast growth factor contained in gelatin hydrogel microspheres on experimental osteoarthritis in the rabbit knee: *Arthritis Rheum.*, Vol. 54, pp. 264-270.
- [22] Jizomoto, H. Kanaoka, E. Sugita, K. & Hirano, K. (1993), Gelatin-acacia microcapsules for trapping micro oil droplets containing lipophilic drugs and ready disintegration in the gastrointestinal tract: *Pharm. Res.*, Vol. 10, pp. 1115-1122.
- [23] Kanyama, M. Kuboki, T. Kojima, S. Fujisawa, T. Hattori, T. Takigawa, M. & Yamashita, A. (2000), Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids of patients with temporomandibular joint osteoarthritis: *J Orofac. Pain.*, Vol. 14, pp. 20-30.
- [24] Kerins, C. A. Carlson, D. S. Hinton, R. J. Grogan, D. M. Marr, K. Kramer, P. R. Spears, R. D. & Bellinger, L. L. (2005), Specificity of meal pattern analysis as an animal model of dermining temporomandibular joint inflammation/pain: *International Journal of Oral Maxillofacial Surgery*, Vol. 34, pp. 425-431.

- [25] Kerins, C. A. Carlson, D. S. McIntosh, J. E. & Bellinger, L. L. (2003), Meal pattern changes associated with temporomandibular joint inflammation/pain in rats; analgesic effects: *Pharmacology Biochemistry and Behavior*, Vol. 75, pp. 181-189.
- [26] Kerins, C. Carlson, D. McIntosh, J. & Bellinger, L. (2004), A role for cyclooxygenase II inhibitors in modulating temporomandibular joint inflammation from a meal pattern analysis perspective: *J Oral Maxillofac Surg*, Vol. 62, pp. 989-995.
- [27] Kramer, P. R. & Bellinger, L. L. (2009), The effects of cycling levels of 17beta-estradiol and progesterone on the magnitude of temporomandibular joint-induced nociception: *Endocrinology*, Vol. 150, pp. 3680-3689.
- [28] Liggins, R. T. Cruz, T. Min, W. Liang, L. Hunter, W. L. & Burt, H. M. (2004), Intra-articular treatment of arthritis with microsphere formulations of paclitaxel: biocompatibility and efficacy determinations in rabbits: *Inflamm.Res*, Vol. 53, pp. 363-372.
- [29] Lin, Y. Weisdorf, D. J. Solovey, A. & Hebbel, R. P. (2000), Origins of circulating endothelial cells and endothelial outgrowth from blood: *J.Clin.Invest*, Vol. 105, pp. 71-77.
- [30] Lu, Y. Zhang, G. Sun, D. & Zhong, Y. (2007), Preparation and evaluation of biodegradable flubiprofen gelatin micro-spheres for intra-articular administration: *J Microencapsul.*, Vol. 24, pp. 515-524.
- [31] Manfredini, D. Chiappe, G. & Bosco, M. (2006), Research diagnostic criteria for temporomandibular disorders (RDC/TMD) axis I diagnoses in an Italian patient population: *J Oral Rehabil.*, Vol. 33, pp. 551-558.
- [32] Mercadante, S. (1999), Problems of long-term spinal opioid treatment in advanced cancer patients: *Pain*, Vol. 79, pp. 1-13.
- [33] Mountziaris, P. M. Kramer, P. R. & Mikos, A. G. (2009), Emerging intra-articular drug delivery systems for the temporomandibular joint: *Methods*, Vol. 47, pp. 134-140.
- [34] Mukherjee, D. Nissen, S. E. & Topol, E. J. (2001), Risk of cardiovascular events associated with selective COX-2 inhibitors: *JAMA: The Journal of the American Medical Association*, Vol. 286, pp. 954-959.
- [35] Plesh, O. Sinisi, S. E. Crawford, P. B. & Gansky, S. A. (2005), Diagnoses based on the Research Diagnostic Criteria for Temporomandibular Disorders in a biracial population of young women: *J Orofac.Pain.*, Vol. 19, pp. 65-75.
- [36] Puebla, P. Pastoriza, P. Barcia, E. & Fernandez-Carballido, A. (2005), PEG-derivative effectively modifies the characteristics of indomethacin-PLGA microspheres destined to intra-articular administration: *J Microencapsul.*, Vol. 22, pp. 793-808.
- [37] Ren, K. (1999), An improved method for assessing mechanical allodynia in the rat: *Physiology and Behavior*, Vol. 67, pp. 711-716.
- [38] Saito, M. Takahashi, K. A. Arai, Y. Inoue, A. Sakao, K. Tonomura, H. Honjo, K. Nakagawa, S. Inoue, H. Tabata, Y. & Kubo, T. (2009), Intraarticular administration of platelet-rich plasma with biodegradable gelatin hydrogel microspheres prevents osteoarthritis progression in the rabbit knee: *Clin Exp Rheumatol.*, Vol. 27, pp. 201-207.
- [39] Srinivas, R. Sorsa, T. Tjaderhane, L. Niemi, E. Raustia, A. Pernu, H. Teronen, O. & Salo, T. (2001), Matrix metalloproteinases in mild and severe temporomandibular joint internal derangement synovial fluid: *Oral Surg.Oral Med Oral Pathol.Oral Radiol.Endod.*, Vol. 91, pp. 517-525.

- [40] Sugisaki, M. Ikai, A. & Tanabe, H. (1995), Dangerous angles and depths for middle ear and middle cranial fossa injury during arthroscopy of the temporomandibular joint.
- [41] Swift, J. Q. Roszkowski, M. T. Alton, T. & Hargreaves, K. M. (1998), Effect of intra-articular versus systemic anti-inflammatory drugs in a rabbit model of temporomandibular joint inflammation: *J Oral Maxillofac Surg*, Vol. 56, pp. 1288-1295.
- [42] Tanaka, E. Detamore, M. S. & Mercuri, L. G. (2008), Degenerative disorders of the temporomandibular joint: etiology, diagnosis, and treatment: *J Dent Res*, Vol. 87, pp. 296-307.
- [43] Thut, P. D. Hermanstyn, T. O. Flake, N. M. & Gold, M. S. (2007), An operant conditioning model to assess changes in feeding behavior associated with temporomandibular joint inflammation in the rat: *J Orofac.Pain*, Vol. 21, pp. 7-18.
- [44] Tuncay, M. Calis, S. Kas, H. S. Ercan, M. T. Peksoy, I. & Hincal, A. A. (2000), Diclofenac sodium incorporated PLGA (50:50) microspheres: formulation considerations and in vitro/in vivo evaluation: *Int.J Pharm.*, Vol. 195, pp. 179-188.
- [45] West, M. J. & Slomanka, L. (2001), 2-D versus 3-D cell counting--a debate. What is an optical disector?: *Trends Neurosci.*, Vol. 24, pp. 374-380.
- [46] Westesson, P. L. Eriksson, L. & Liedberg, J. (1986), The risk of damage to facial nerve, superficial temporal vessels, disk, and articular surfaces during arthroscopic examination of the temporomandibular joint.
- [47] Yoshida, K. Takatsuka, S. Hatada, E. Nakamura, H. Tanaka, A. Ueki, K. Nakagawa, K. Okada, Y. Yamamoto, E. & Fukuda, R. (2006), Expression of matrix metalloproteinases and aggrecanase in the synovial fluids of patients with symptomatic temporomandibular disorders: *Oral Surg.Oral Med Oral Pathol.Oral Radiol.Endod.*, Vol. 102, pp. 22-27.
- [48] Zuniga, J. R. Ibanez, C. & Kozacko, M. (2007), The analgesic efficacy and safety of intra-articular morphine and mepivacaine following temporomandibular joint arthroplasty: *J Oral Maxillofac Surg*, Vol. 65, pp. 1477-1485.

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The purpose of this book was to offer an overview of recent insights into the current state of arthroplasty. The tremendous long term success of Sir Charnley's total hip arthroplasty has encouraged many researchers to treat pain, improve function and create solutions for higher quality of life. Indeed and as described in a special chapter of this book, arthroplasty is an emerging field in the joints of upper extremity and spine. However, there are inborn complications in any foreign design brought to the human body. First, in the chapter on infections we endeavor to provide a comprehensive, up-to-date analysis and description of the management of this difficult problem. Second, the immune system is faced with a strange material coming in huge amounts of micro-particles from the tribology code. Therefore, great attention to the problem of aseptic loosening has been addressed in special chapters on loosening and on materials currently available for arthroplasty.

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