# Evidence of Silicon Dioxide Crystals in Synovial Fluid of Patients with Osteoarthritis

FRANCESCA OLIVIERO, PAOLA FRALLONARDO, LUCA PERUZZO, ROBERTA RAMONDA, PAOLO SFRISO, ANNA SCANU, LEONARDO TAURO, FRÉDÉRIC LIOTÉ, and LEONARDO PUNZI

*ABSTRACT.* ***Objective.*** Synovial fluid (SF) may contain a number of crystals that optical microscopy is unable to identify with certainty. Scanning electron microscopy (SEM) was utilized in this study to characterize SF crystals in the context of knee osteoarthritis (OA).

***Methods.*** SF was collected from the knees of 25 patients with OA and examined under optical light microscopy. Calcium pyrophosphate dihydrate (CPPD) crystals were assessed by means of compensated polarized light microscopy, while alizarin red S staining was performed to identify apatite (BCP) crystals. All the specimens were also analyzed by SEM and x-ray diffractometry, as gold standards.

***Results.*** CPPD crystals were found in 32% and BCP in 24% of the SF examined by SEM. The degree of concordance between polarized light microscopy and SEM was 0.83 for CPPD and 0.46 for BCP (kappa statistic). The secondary and backscatter electron SEM observations allowed identification of silicon dioxide ($SiO_2$) crystals in 8 out of 10 patients in whom polarized light microscopy revealed irregular and polymorph crystals.

***Conclusion.*** $SiO_2$ crystals cannot be readily identified by their morphology or polarization properties under optical microscopy. Their presence, nevertheless, did not lead to misclassification. (First Release April 15 2008; J Rheumatol 2008;35:1092–5)

*Key Indexing Terms:*
SYNOVIAL FLUID OSTEOARTHRITIS CALCIUM PYROPHOSPHATE
CALCIUM PHOSPHATE SILICON DIOXIDE

Calcium crystals are common features in osteoarthritis (OA). Their exact significance in synovial fluid (SF), however, is unclear. While calcium pyrophosphate dihydrate (CPPD) crystals are often found in OA, they also define unique clinical subsets of patients with inflammatory arthritis[1]. Their identification is widely postulated as the "gold standard" for the diagnosis of CPPD deposition disease[2]. Actively involved in tissue damage and considered markers of severe OA, basic calcium phosphate (BCP) crystals are even more common than CPPD in OA joints[3]. Detection of calcium crystals in SF is therefore an important part of patient management.

Identification of SF crystals in clinical practice is made possible by polarized light microscopy, a simple, affordable procedure[4]. One of its major drawbacks is its lack of specificity, in part attributable to observer misinterpretation[5]. On the other hand, SF may contain a number of crystals and other particulate matter that even a trained physician or technician is unable to identify with complete certainty under optical microscopy.

In our study, scanning electron microscopy (SEM) was utilized to characterize some of these types of crystals and to evaluate their possible clinical significance with respect to OA.

## MATERIALS AND METHODS

*Patients and samples.* SF was collected by arthrocentesis from the knees of 25 patients diagnosed with OA (17 women, 8 men, mean age 67.4 ± 9.4 yrs) referred to the outpatient clinics of the Division of Rheumatology of the University of Padova. None of these patients had undergone intraarticular steroid or sodium hyaluronate injections for at least 3 months prior to the examination.

*SF analysis by optical microscopy.* SF samples were promptly examined following arthrocentesis under optical light microscopy. The total white blood cell (WBC) count was measured using a standard hematological counting chamber. A differential cell count was carried out using supravital staining (Testsimplets, Waldek®). CPPD crystals were assessed after centrifugation on the basis of their shape as well as the degree and sign of birefringence by means of compensated polarized light microscopy, while alizarin red S staining was performed to identify BCP crystals, which precipitate forming red birefringent and circular complexes[6].

*From the Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy.*

*F. Oliviero, PhD; P. Frallonardo, MD, Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Padova; L. Peruzzo, PhD, Institute of Geosciences and Georesources, CNR Padova; R. Ramonda, MD, PhD; P. Sfriso, MD, PhD; A. Scanu, PhD, Department of Clinical and Experimental Medicine, University of Padova; L. Tauro, Department of Geosciences, University of Padova; F. Lioté, MD, PhD, Rheumatology Federation, Viggo Petersen Center, INSERM U606, Lariboisière Teaching Hospital, Paris, France; L. Punzi, MD, PhD, Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Padova.*

*Address reprint requests to F. Oliviero, Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Padova, Via Giustiniani 2, 35128 Padova, Italy. E-mail: francesca.oliviero@unipd.it*

*Accepted for publication January 4, 2008.*

*SF analysis by SEM and X-ray diffractometry.* All specimens were carefully prepared using the procedures outlined here. The samples were manipulated with care to avoid any external contamination. Glass equipment was never used. Blank samples of deionized water were prepared similarly and analyzed as a negative control.

Five milliliters of each sample were stored after arthrocentesis to be used when the crystals were separated from the liquid. The samples were centrifuged 3 times, 5 min each, at a speed of 3,000 rpm. After each centrifugation the upper liquid phase was extracted and replaced with deionized water. The crystals concentrated at the bottom of the tube were extracted and placed on an adhesive carbon disc and oven-dried at 30°C for 24 h. The samples were then carbon-coated in preparation for analysis by SEM and by x-ray diffractometry.

*Statistical analysis.* The Mann-Whitney U test was performed to evaluate differences among groups positive to CPPD, BCP, and $SiO_2$ crystals by SEM. The degree of concordance between optical microscopy and SEM was evaluated by Cohen's kappa statistic. Data were analyzed with SPSS software (v. 14.0; SPSS, Chicago, IL, USA); a p value < 0.05 was considered significant.

## RESULTS

The patients' clinical data and SF features are summarized in Table 1. The SF presented the following features [median (range)]: volume 30 ml (1–150), WBC 100 cells/$mm^3$ (100–800), and polymorphonuclear cells 2% (2%–22%). Analysis under polarized light revealed CPPD crystals in 16% of all SF, while BCP crystals were suspected in 60% of the samples because of the presence of glossy chunks positively stained by red alizarin (Table 2). Irregular, polymorphic, extracellular, and weakly birefringent crystals were observed in 40% of SF. When the same samples were examined by SEM, CPPD crystals were present in 32% and BCP in 24% of SF (Table 2). Both types of pathogenic crystals were identified in 12% of SF. By kappa statistic, the degree of concordance between polarized light microscopy and SEM was 0.83 for CPPD and 0.46 for BCP.

The secondary and backscatter electron SEM observations also permitted identification of silicon dioxide crystals (Figure 1) in 8 out of 10 patients in whom polarized light microscopy revealed irregular, polymorphic crystals. The x-ray diffractometry spectra confirmed the crystalline phase of $SiO_2$ (Figure 1). One of these patients also had CPPD crys-

*Table 2.* Presence of CPPD and BCP crystals according to optical microscopy (OM) and scanning electron microscopy (SEM) analysis.

| | OM, n (%) | SEM, n (%) | k |
|---|---|---|---|
| CPPD | 4 (16) | 8 (32) | 0.83 |
| BCP | 15 (60) | 6 (24) | 0.46 |
| CPPD + BCP | 3 (12) | 3 (12) | |
| Other | 10 (40) | 8 (32) | |

k: concordance coefficient.

*Table 1.* Clinical data and synovial fluid features of patients studied. Patients are numbered on the basis of the presence of crystals.

| Patient | Sex | Age, yrs | Disease Duration, yrs | Synovial Fluid Features: Volume, ml | WBC, per $mm^3$ | PMN, % | Optical Microscopy: CPPD | Alizarin Stain | Other Crystals | Scanning Electron Microscopy: CPPD | BCP | $SiO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 55 | 3 | 30 | 100 | 2 | – | + | + | – | – | + |
| 2 | M | 66 | 15 | 10 | 300 | 2 | – | + | + | – | – | + |
| 3 | M | 66 | 14 | 20 | 100 | 2 | – | – | + | – | – | + |
| 4 | F | 62 | 1 | 30 | 100 | 4 | – | + | + | – | – | + |
| 5 | F | 76 | 9 | 30 | 100 | 4 | – | + | + | – | – | + |
| 6 | M | 58 | 1 | 45 | 600 | 10 | – | – | + | – | – | + |
| 7 | F | 76 | 6 | 4 | 100 | 4 | + | + | + | + | – | + |
| 8 | F | 60 | 4 | 20 | 400 | 2 | – | – | + | – | – | + |
| 9 | M | 73 | 5 | 30 | 500 | 2 | – | ++ | – | + | + | – |
| 10 | M | 65 | 4 | 10 | 100 | 2 | – | +++ | + | + | + | – |
| 11 | F | 80 | 10 | 80 | 800 | 16 | – | – | – | + | – | – |
| 12 | F | 63 | 8 | 4 | 100 | 2 | – | ++ | – | – | + | – |
| 13 | M | 78 | 20 | 20 | 600 | 2 | + | + | – | + | – | – |
| 14 | F | 57 | 5 | 1 | 100 | 2 | – | + | – | – | + | – |
| 15 | F | 69 | 3 | 35 | 300 | 22 | + | + | – | – | + | – |
| 16 | F | 80 | 10 | 50 | 600 | 8 | – | ++ | + | + | + | – |
| 17 | M | 61 | 6 | 150 | 100 | 2 | + | – | – | + | – | – |
| 18 | F | 75 | 5 | 30 | 700 | 2 | – | – | – | + | – | – |
| 19 | F | 69 | 6 | 6 | 300 | 2 | – | ++ | – | – | – | – |
| 20 | F | 66 | 10 | 80 | 300 | 6 | – | – | – | – | – | – |
| 21 | F | 58 | 7 | 30 | 100 | 2 | – | – | – | – | – | – |
| 22 | F | 45 | 2 | 40 | 100 | 2 | – | – | – | – | – | – |
| 23 | F | 78 | 10 | 10 | 100 | 10 | – | + | – | – | – | – |
| 24 | F | 83 | 12 | 30 | 100 | 2 | – | + | – | – | – | – |
| 25 | M | 68 | 2 | 12 | 100 | 2 | – | – | – | – | – | – |

WBC: white blood cell count; PMN: polymorphonuclear leukocytes; CPPD: calcium pyrophosphate dihydrate; BCP: basic calcium phosphate.

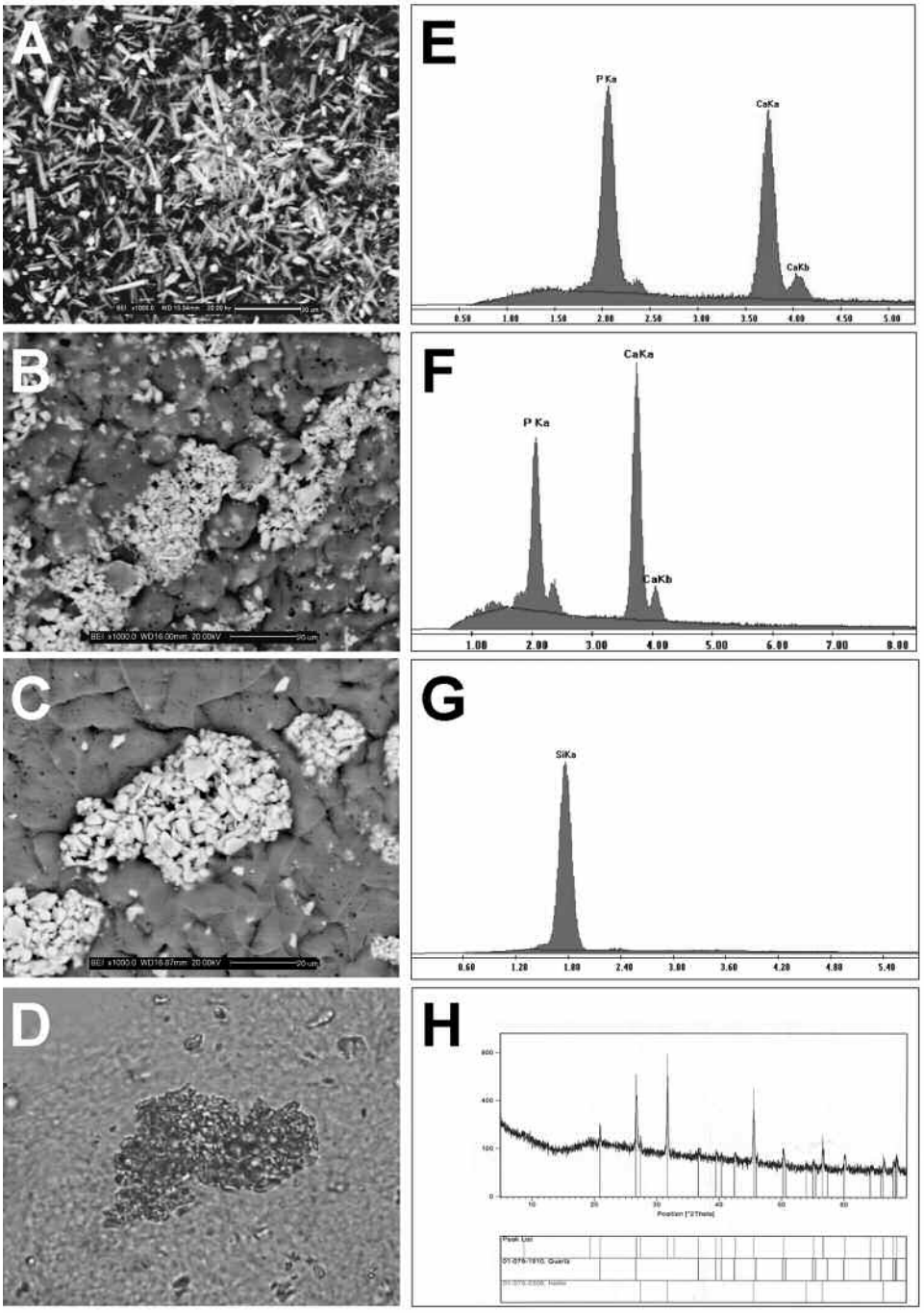


*Figure 1.* CPPD (A), BCP (B) and $SiO_2$ (C) crystals detected by SEM with their x-ray emission spectra (E, F, G). Irregular crystals detected by ordinary light microscopy (D; original magnification 1000×) in a patient positive for $SiO_2$ by SEM. X-ray diffraction spectrum of $SiO_2$ is shown (H).

tals. No $SiO_2$ crystals were detected in the blank samples of deionized water utilized as a negative control.

Univariate analysis (Mann-Whitney U test) was performed according to the presence/absence of each type of crystal by SEM. It was found that patients positive for CPPD crystals were older (mean 74.7 ± 6.5 yrs vs 64.4 ± 9.2 yrs; p = 0.03) and showed a higher WBC count (437.5 ± 292.4 cells/$mm^3$ vs 194.1 ± 147.7 cells/$mm^3$; p = 0.04).

## DISCUSSION

SEM was utilized to further characterize SF crystals in the context of knee OA. CPPD crystals were found in 32% and

BCP in 24% of the SF examined. SEM also permitted $SiO_2$ crystals to be detected in 32% of the patients. $SiO_2$ crystals cannot be identified by their morphology or polarization properties under optical microscopy. While their presence did not lead to misclassification, their identification seems, nevertheless, to be irrelevant to clinical practice.

The origin of silicon dioxide crystals in joint cavities is still uncertain. Silicon-containing particles have been described in SF as possible artefacts produced by technical procedures[7,8]. We were able to rule out this possibility by utilizing the same procedures to prepare and analyze blank samples of deionized water.

While x-ray spectra of SF specimens were capable of detecting Si and O, it was unclear which crystalline phase (quartz, tridymite, cristobalite, etc.) or amorphous silica (glass, opal, etc.) was involved. This question was answered by means of x-ray diffractometry, the most appropriate technique for determining types of mineral species from a powder. The position and intensity of the x-ray diffraction peaks provide a fingerprint of each crystalline material if sufficiently represented in the powder. The x-ray diffractometry spectra obtained from SF samples unequivocally displayed the sharp peaks of a crystalline phase corresponding to quartz.

Not consistent with previous studies hypothesizing that $SiO_2$ crystals have an extrinsic origin, the implications of our findings are uncertain[8]. That silicon is required for growth and for bone cartilage formation is well known, and it is thought to act as a biological crosslinking agent in the formation of connective tissue. Silicon contributes to cartilage architecture and resilience by means of an interlacing structure between mucopolysaccharides and proteins[9].

Future studies may be able to clarify how $SiO_2$ crystals form in SF, and suggest their longterm consequences with respect to progression of OA.

## REFERENCES


1. Rosenthal AK. Update in calcium deposition diseases. Curr Opin Rheumatol 2007;19:158-62.
2. Chen LX, Schumacher HR. Current trends in crystal identification. Curr Opin Rheumatol 2006;18:171-3.
3. Whelan L, Whelan LC, Morgan MP, McCarthy GM. Basic calcium phosphate crystals as a unique therapeutic target in osteoarthritis. Front Biosci 2005;10:530-41.
4. Dieppe P, Swan A. Identification of crystals in synovial fluid. Ann Rheum Dis 1999;58:261-3.
5. Lumbreras B, Pascual E, Frasquet J, Gonzalez-Salinas J, Rodriguez E, Hernandez-Aguado I. Analysis for crystals in synovial fluid: training of the analysts results in high consistency. Ann Rheum Dis 2005;64:612-5.
6. Paul H, Reginato AJ, Schumacher HR. Alizarin red S staining as a screening test to detect calcium compounds in synovial fluid. Arthritis Rheum 1983;26:191-200.
7. Bardin T, Schumacher HR, Lansaman J, Rothfuss S, Dryll A. Transmission electron microscopic identification of silicon-containing particles in synovial fluid: potential confusion with calcium pyrophosphate dihydrate and apatite crystals. Ann Rheum Dis 1984;43:624-7.
8. Faure G, Netter P, Bene MC. Silicon-containing particles in synovial fluid: scanning electron microscopy coupled with analytical techniques allows an easy identification and differentiation from pathologically relevant crystals. Ann Rheum Dis 1985;44:148-50.
9. Schwarz K. A bound form of silicon in glycosaminoglycans and polyuronides. Proc Natl Acad Sci USA 1973;70:1608-12.