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Sartika Puspita, Marsetyawan HNE Soesatyo, Siti Sunarintyas, and Ema Mulyawati



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The Fibroin (*Bombyx mori* L.) is Cytocompatible with Human Primary Pulp Cells

Sartika Puspita^{1, 2, a)}, Marsetyawan HNE Soesatyo³,
Siti Sunarintyas⁴, and Ema Mulyawati⁵

¹Faculty of Dentistry, Universitas Gadjah Mada, Jl. Denta 1, Sekip Utara, Yogyakarta 55281 Indonesia.

²Department of Oral Biology, School of Dentistry, Universitas Muhammadiyah,
Jl. Brawijaya, Kasihan, Bantul, Yogyakarta 55183, Indonesia.

³Department of Histology and Cells Biology, Faculty of Medicine, Public Health and Nursing,
Universitas Gadjah Mada, Jl. Farmako, Sekip Utara, Yogyakarta, 55281, Indonesia.

⁴Department of Dental Biomaterial, Faculty of Dentistry, Universitas Gadjah Mada,
Jl. Denta 1, Sekip Utara, Yogyakarta, 55281, Indonesia.

⁵Department of Conservative Dentistry and Endodontic, Faculty of Dentistry, Universitas Gadjah Mada,
Jl. Denta 1, Sekip Utara, Yogyakarta, 55281, Indonesia.

^{a)}Corresponding author: sartika.puspita@umy.ac.id

Abstract. Fibroin cocoons (*Bombyx mori* L.) attracts many researchers to undertake various studies especially in the field of medicine for example as suturing materials, bone graft, and artificial ligaments because they contain proteins consisting of 18 amino acids. Further research still can be done in the field of dentistry including the application of fibroin as pulp capping materials. In this study, we investigate the cytocompatibility of *B. mori* fibroin cocoon by determining the viability of dental pulp cells to some concentrations of fibroin extract. Since in the future fibroin would be used as one of pulp capping material. The method of this study was laboratory experimental in vitro using primary pulp cells of human teeth that were extracted for orthodontic purposes. The concentration of fibroin was $100 \mu\text{g} \cdot \text{mL}^{-1}$; $50 \mu\text{g} \cdot \text{mL}^{-1}$; $25 \mu\text{g} \cdot \text{mL}^{-1}$; $12.5 \mu\text{g} \cdot \text{mL}^{-1}$; $6.25 \mu\text{g} \cdot \text{mL}^{-1}$; $3.125 \mu\text{g} \cdot \text{mL}^{-1}$ and $1.56 \mu\text{g} \cdot \text{mL}^{-1}$. 96 well plates of MTT assay and absorbance values were determined by ELISA reader with 595 nm wavelength. Moreover, the response of pulp cell is calculated with the formula. The results show that the viability of fibroblast pulp cells at concentration $100 \mu\text{g} \cdot \text{mL}^{-1}$ was 104 %, $50 \mu\text{g} \cdot \text{mL}^{-1}$ was 108 %, $25 \mu\text{g} \cdot \text{mL}^{-1}$ was 114 %, $12.5 \mu\text{g} \cdot \text{mL}^{-1}$ was 120 %, $6.25 \mu\text{g} \cdot \text{mL}^{-1}$ was 116 %, $3.125 \mu\text{g} \cdot \text{mL}^{-1}$ was 112 %, and $1.56 \mu\text{g} \cdot \text{mL}^{-1}$ was 92 %. The conclusion of this research is the viability of pulp cells to fibroin among 92 % to 120 %. Further analysis, the concentration of fibroin does not influence the viability of cells, which mean fibroin is cytocompatible with human pulp cells.

Keywords: *Bombyx mori* L., Central Java, cytocompatibility, fibroin cocoons, pulp cells.

INTRODUCTION

Direct pulp capping is the endodontic treatment to maintain pulp vitality and decrease the need for root canal treatment. The expose pulp must cover the pulp capping material in order to induce differentiation of mesenchymal pulp cells to form odontoblast-like cells to produce reparative dentin. Then, reparative dentin as a hard barrier, avoid pulp from exposure. Therefore, its biological function and vitality of the pulp protected [1]. One of the requirements of an ideal endodontics repair material ideally good biocompatibility and bioactivity to promote reparative dentinogenesis [2]. Several studies presented the success of various kinds of direct pulp capping material, including calcium hydroxide, hydrophilic resins, resin-modified glass ionomer cement, tricalcium phosphates, and mineral trioxide aggregate (MTA). Success rates range from 30 % to 85 % in 2 yr to 10 yr retrospective studies [3]. There has some disagreement concern in the success of treatment thus the research on pulp capping material is still at work [3].

We have found an innovative method to find new material of pulp capping. The use of a biological molecule is considered to induce reparative dentin formation with structural properties better than the present.

Fibroin is a natural macromolecule derived from silkworm cocoons (*Bombyx mori*) that attract scientists from various disciplines to conduct various studies [4]. Fibroin has a long history of use in the medicine, for example as suturing materials, bone graft, and artificial ligaments. Fibroin is a protein consisting of 18 amino acids [5]. They were glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, glutamic acid, lysine, arginine, histidine, tyrosine, phenylalanine, proline, tryptophan, methionine, and cysteine-2 [6]. Some studies claim fibroin has adhesion function to collagen substrate and increase the growth of bone tissue in the bone graft treatment. During these two decades, fibroin derived protein is widely used as materials for research and development of biomaterial and biomedical material [4].

The molecular crystal structure of *B. mori* silk fibroin peptide determined by the X-ray diffraction method shows the same cell dimension structure as the polypeptide (L-Ala-Gly). Based on molecular observations, fibroin has the form of "crankshaft" or "S-shape zig-zag" is arranged based on the number of multiples dipeptide sequence and hexapeptide also hydrogen bonds [7]. The crystalline structure of fibroin based on X-ray analysis shows the peptide bond in the form of a widening bond can be seen in Fig. 1.

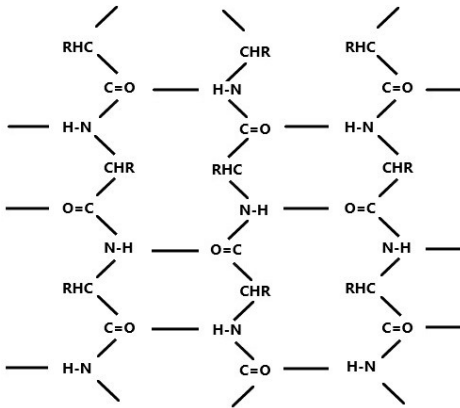


FIGURE 1. The crystalline structure of the silk fibroin peptide chain of *Bombyx mori* [7]

Many researchers inform the advantages of silk fibroin [8]. There are biocompatible, biodegradable, stable, and it has mechanical and functional strength for biomedical applications [5]. However, there is no study about cytocompatibility of fibroin to primary human dental pulp cells.

MATERIALS AND METHODS

Bombyx mori cocoon obtained from Silkworm Cultivation Centre, Central Java, Indonesia. The method of this study was laboratory experimental in vitro using primary pulp cells of human teeth were extracted for orthodontic purposes. The patients agreed and signed informed consent. The research conducted to Medical Ethics of Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia.

The MTT assay was performed to measure the activity of enzymes that reduce MTT (SIGMA-Aldrich, St. Louis, MO, USA) and convert it to formazan dye, creating a purple colour. This application to assess the cytocompatibility, cell viability, and proliferation. Also, it can be used to determine the cytotoxicity of pharmaceutical agents or toxic compounds since these materials prevent cell proliferation. The cells were placed in 96-well plates in such a way that each well contained 5 000 cells and 100 μL of the respective material. After 24 h, the cells were exposed to fibroin cocoon of *B. mori* L. Fibroin concentrations used were 100 $\mu\text{g} \cdot \text{mL}^{-1}$, 50 $\mu\text{g} \cdot \text{mL}^{-1}$, 25 $\mu\text{g} \cdot \text{mL}^{-1}$, 12.5 $\mu\text{g} \cdot \text{mL}^{-1}$, 6.25 $\mu\text{g} \cdot \text{mL}^{-1}$, 3.125 $\mu\text{g} \cdot \text{mL}^{-1}$ and 1.56 $\mu\text{g} \cdot \text{mL}^{-1}$. Then the absorbance values were determined to

use ELISA reader with 595 nm wavelength (Biotek, Winoosky, VT, United States), and the cell viability is calculated to use the formula [8].

RESULTS AND DISCUSSION

The viability of fibroblast pulp cells at concentrations of $100 \mu\text{g} \cdot \text{mL}^{-1}$ was 104 %; $50 \mu\text{g} \cdot \text{mL}^{-1}$ was 108 %; $25 \mu\text{g} \cdot \text{mL}^{-1}$ was 114 %; $12.5 \mu\text{g} \cdot \text{mL}^{-1}$ was 120 %; $6.255 \mu\text{g} \cdot \text{mL}^{-1}$ was 116 %; $3.125 \mu\text{g} \cdot \text{mL}^{-1}$ was 112 %, and $1.56 \mu\text{g} \cdot \text{mL}^{-1}$ was 92 % as seen on the Fig. 2.

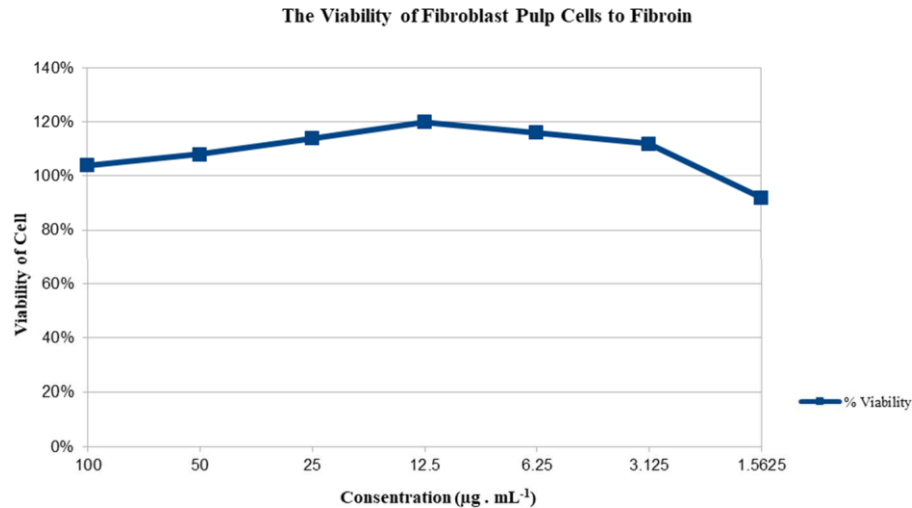


FIGURE 2. The viability of primary human dental pulp cells to *Bombyx mori* fibroin cocoon

Cytocompatibility of *B. mori* fibroin needs to assess because every new biomaterial has a potential risk of human safety. One of the international standards gives a suggestion for *in vitro* assessment [8]. This research is a preliminary study to investigate the viability of human pulp fibroblast cells with fibroin. Figure 2 shows the viability of cells above 100 % almost at all concentrations. It can be interpreted fibroin has cytocompatibility to human pulp cells. One of the requirements of the new material can be applied to the human tissue is biocompatibility. Based on a previous study that examined sericin of *B. mori* cocoon has higher biocompatibility than other types of cocoon silk which is $230 \text{ mg} \cdot \text{mL}^{-1}$ [8]. It means *B. mori* cocoon has better biocompatibility than other silk despite low biocompatibility of sericin than fibroin. The previous study reports the viability of human primary fibroblast cells just only 64.486 % in the $100 \mu\text{g} \cdot \text{mL}^{-1}$ concentration of sericin [8].

Several studies declare fibroin has osteogenesis effect as bone graft material [9–11]. Recently, it has been developed as an artificial corneal in tissue engineering appliance [12] and fibroin solution is being studied as eye drops to stimulate epithelial cell proliferation and attachment [13]. All the studies mentioned above are being developed and based on references still *in vitro* and *in vivo* studies. Based on these studies, the cytocompatibility of fibroin on dental pulp fibroblasts can give new information in the progress of *B. mori* fibroin research.

According to the MTT Assay test due to cytocompatibility of fibroin on dental pulp cells, fibroin can be continued with further research to conduct *in vivo* studies on experimental animal teeth. Fibroin is a candidate for direct pulp capping material because of the good biocompatibility. Fibroin can maintain fibroblast pulp cells alive in the highest concentration.

CONCLUSION

The conclusion of this study was the *Bombyx mori* fibroin is cytocompatible to human pulp cells.

ACKNOWLEDGMENT

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The Title Goes Here with Each Initial Letter Capitalized

Author's Name,^{1, a)} Author's Name,^{1, b)} and Another's Name^{2, 3, c)}

¹⁾ Replace this text with an author's affiliation (use complete addresses, including country name or code).

²⁾ Second institution and/or address

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^{a)} Corresponding author: `first.author@institution.edu`

^{b)} Electronic mail: `second.author@institution.edu`.

^{c)} Electronic mail: `third.author@anotherinstitution.edu`

Abstract. An article usually includes an abstract, a concise summary of the work covered at length in the main body of the article. It is used for secondary publications and for information retrieval purposes.

FIRST-LEVEL HEADING: THE LINE BREAK WAS FORCED via \\

This sample document demonstrates proper use of REVTeX 4.1 (and L^ATeX 2 ϵ) in manuscripts prepared for submission to AIP conference proceedings. Further information can be found in the documentation included in the distribution or available at <http://authors.aip.org> and in the documentation for REVTeX 4.1 itself.

When commands are referred to in this example file, they are always shown with their required arguments, using normal TeX format. In this format, #1, #2, etc. stand for required author-supplied arguments to commands. For example, in `\section{#1}` the #1 stands for the title text of the author's section heading, and in `\title{#1}` the #1 stands for the title text of the paper.

Line breaks in section headings at all levels can be introduced using `\\`. A blank input line tells TeX that the paragraph has ended.

Second-level heading: Formatting

This file may be formatted in both the `preprint` (the default) and `reprint` styles; the latter format may be used to mimic final journal output. Either format may be used for submission purposes. Hence, it is essential that authors check that their manuscripts format acceptably under `preprint`. Manuscripts submitted to AIP that do not format correctly under the `preprint` option may be delayed in both the editorial and production processes.

Third-level heading: Citations and Footnotes

Citations in text refer to entries in the Bibliography; they use the commands `\cite{#1}` or `\onlinecite{#1}`. Because REVTeX uses the `natbib` package of Patrick Daly, its entire repertoire of commands are available in your document; see the `natbib` documentation for further details. The argument of `\cite` is a comma-separated list of *keys*; a key may consist of letters and numerals.

By default, citations are numerical; [1] author-year citations are an option. To give a textual citation, use `\onlinecite{#1}`: (Refs. 2, 3, and 4). REVTeX “collapses” lists of consecutive numerical citations when appropriate. REVTeX provides the ability to properly punctuate textual citations in author-year style; this facility works correctly with numerical citations only with `natbib`'s `compress` option turned off. To illustrate, we cite several together [1, 2, 3, 5], and once again (Refs. 1, 3, 4, and 5). Note that, when numerical citations are used, the references were sorted into the same order they appear in the bibliography.

A reference within the bibliography is specified with a `\bibitem{#1}` command, where the argument is the citation key mentioned above. `\bibitem{#1}` commands may be crafted by hand or, preferably, generated by using BibTeX. The AIP styles for REVTeX 4 include BibTeX style files `aipnum.bst` and `aipauth.bst`, appropriate for numbered and author-year bibliographies, respectively. REVTeX 4 will automatically choose the style appropriate for

the document's selected class options: the default is numerical, and you obtain the author-year style by specifying a class option of `author-year`.

This sample file demonstrates a simple use of the BibTeX tool via a `\bibliography` command referencing the `aipsamp.bib` file. Running BibTeX (in this case `bibtex aipsamp`) after the first pass of L^AT_EX produces the file `aipsamp.bbl` which contains the automatically formatted `\bibitem` commands (including extra markup information via `\bibinfo` commands). If not using BibTeX, the `thebibliography` environment should be used instead.

Fourth-level heading is run in. Footnotes are produced using the `\footnote{#1}` command. Numerical style citations put footnotes into the bibliography [6]. Author-year and numerical author-year citation styles (each for its own reason) cannot use this method. Note: due to the method used to place footnotes in the bibliography, *you must re-run BibTeX every time you change any of your document's footnotes.*

MATH AND EQUATIONS

Inline math may be typeset using the `$` delimiters. Bold math symbols may be achieved using the `bm` package and the `\bm{#1}` command it supplies. For instance, a bold α can be typeset as `\$ \bm{\alpha} \$` giving α . Fraktur and Blackboard (or open face or double struck) characters should be typeset using the `\mathfrak{#1}` and `\mathbb{#1}` commands respectively. Both are supplied by the `amssymb` package. For example, `\$ \mathbb{R} \$` gives \mathbb{R} and

`\$ \mathfrak{G} \$` gives \mathfrak{G}

In L^AT_EX there are many different ways to display equations, and a few preferred ways are noted below. Displayed math will center by default. Use the class option `fleqn` to flush equations left.

Below we have numbered single-line equations, the most common kind:

$$\chi_+(p) = [2|p|/(|p|+p_z)]^{-1/2} \sum_{\alpha} \sum_{\beta} \dots \quad (1)$$

$$\frac{1}{A} \frac{2}{b} \quad (2)$$

Note the open one in Eq. (2).

Not all numbered equations will fit within the text width this way. The equation number will move down automatically if it cannot fit on the same line with a one-line equation:

$$ab12345678abc123456abcde f a \beta \gamma \delta 1234556a \beta \frac{1}{2} \sum_{\alpha} \sum_{\beta} \dots \quad (3)$$

When the `\label{#1}` command is used [cf. input for Eq. (2)], the equation can be referred to in text without knowing the equation number that T_EX will assign to it. Just use `\ref{#1}`, where `#1` is the same name that used in the `\label{#1}` command.

Unnumbered single-line equations can be typeset using the `\[, \]` format:

$$g^+ g^+ \rightarrow g^+ g^+ g^+ g^+ \dots q^+ q^+ \rightarrow q^+ g^+ g^+ \dots$$

Multiline equations

Multiline equations are obtained by using the `eqnarray` environment. Use the `\nonumber` command at the end of each line to avoid assigning a number:

$$M = i g^2 (A E_1 E_2)^{1/2} (l^2)^{-1} \delta_{\sigma, -\sigma} (g^e)^2 \chi_{\tau \bar{\tau}}(p_2) \quad (4)$$

$$\times [\epsilon_j l_i \epsilon_i]_{\sigma_1} \chi_{\sigma_1}(p_1),$$

$$\sum |M_g^{\text{viol}}|^2 = g^{2n-4} (Q^2)^{N^{n-2}} (N^2 - 1) \quad (5)$$

$$\times \sum_{i \neq j} \sum_{\text{perm}} \sum_{\tau} \frac{1}{s_{12}} \frac{1}{s_{\bar{1}2}} \sum_{\tau} c_{\tau}^f.$$

TABLE I. This table illustrates left-aligned, centered, and right-aligned columns. Note that REVTeX 4 adjusts the intercolumn spacing so that the table fills the entire width of the text. Table captions are numbered automatically.

Left ^a	Centered ^b	Right
1	2	3
10	20	30
100	200	300

^a Note a.

^b Note b.

Note: Do not use `\label{#1}` on a line of a multiline equation if `\nonumber` is also used on that line. Incorrect cross-referencing will result. Notice the use `\text{#1}` for using a Roman font within a math environment.

To set a multiline equation without *any* equation numbers, use the `\begin{eqnarray*}`, `\end{eqnarray*}` format:

$$\sum |M_g^{\text{viol}}|^2 = g^{2n-4} (Q^2)^{N^{m-2}} (N^2 - 1) \times \sum_{i < j} \sum_{\text{perm}} \frac{1}{S_{12} S_{23} S_{n1}} \frac{1}{S_{12}}.$$

To obtain numbers not normally produced by the automatic numbering, use the `\tag{#1}` command, where #1 is the desired equation number. For example, to get an equation number of (5.1^j),

g g → g g g g ... , q q → q g g ...
) A few notes on `\tag{#1}`. `\tag{#1}` requires `amsmath`. The `\tag{#1}` must come before the `\label{#1}`, if any. The numbering set with `\tag{#1}` is *transparent* to the automatic numbering in REVTeX; therefore, the number must be known ahead of time, and it must be manually adjusted if other equations are added. `\tag{#1}` works with both single-line and multiline equations. `\tag{#1}` should only be used in exceptional case - do not use it to number all equations in a paper.

Enclosing single-line and multiline equations in `\begin{subequations}` and `\end{subequations}` will produce a set of equations that are “numbered” with letters, as shown in Eqs. (6a) and (6b) below:

$$abc123456abcde f a \beta \gamma \delta 1234556a \beta \frac{1}{A^2} \sum \quad (6a)$$

$$M = i g^2 (A E_1 E_2)^{1/2} (l^2)^{-1} (g^e)^2 \chi_{\sigma \sigma} (p_2)_2$$

$$\times [\epsilon_i]_{\sigma_i} \chi_{\sigma_i} (p_1). \quad (6b)$$

Putting a `\label{#1}` command right after the `\begin{subequations}`, allows one to reference all the equations in a subequations environment. For example, the equations in the preceding subequations environment were Eqs. (6).

CROSS-REFERENCING

REVTeX will automatically number sections, equations, figure captions, and tables. In order to reference them in text, use the `\label{#1}` and `\ref{#1}` commands. To reference a particular page, use the `\pageref{#1}` command.

The `\label{#1}` should appear in a section heading, within an equation, or in a table or figure caption. The `\ref{#1}` command is used in the text where the citation is to be displayed. Some examples: Section on page 1, Table I, and Fig. 1.

FIGURES AND TABLES

Figures and tables are typically “floats”; L^AT_EX determines their final position via placement rules. L^AT_EX isn’t always successful in automatically placing floats where you wish them.

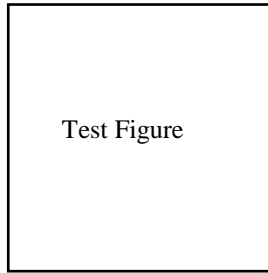


FIGURE 1. A figure caption. The figure captions are automatically numbered.

TABLE II. Numbers in columns Three–Five have been aligned by using the “d” column specifier (requires the `dcolumn` package). Non-numeric entries (those entries without a “.”) in a “d” column are aligned on the decimal point. Use the “D” specifier for more complex layouts.

One	Two	Three	Four	Five
one	two	three	four	five
He	2	2.77234	45672.	0.69
C ^a	C ^b	12537.64	37.66345	86.37

^a Some tables require footnotes.

^b Some tables need more than one footnote.

Figures are marked up with the `figure` environment, the content of which imports the image (`\includegraphics`) followed by the figure caption (`\caption`). The argument of the latter command should itself contain a `\label` command if you wish to refer to your figure with `\ref`.

Import your image using either the `graphics` or `graphicx` packages. Both of these packages define the `\includegraphics{#1}` command, but they differ in the optional arguments for specifying the orientation, scaling, and translation of the figure. Fig. 1 is an example of this.

The analog of the `figure` environment is `table`, which uses the same `\caption` command. However, you should type your caption command first within the `table`, instead of last as you did for `figure`.

The heart of any table is the `tabular` environment, which represents the table content as a (vertical) sequence of table rows, each containing a (horizontal) sequence of table cells. Cells are separated by the `&` character; the row terminates with `\end{tabular}`. The required argument for the `tabular` environment specifies how data are displayed in each of the columns. For instance, a column may be centered (`c`), left-justified (`l`), right-justified (`r`), or aligned on a decimal point (`d`). (Table II illustrates the use of decimal column alignment.)

Extra column-spacing may be specified as well, although REVTeX 4 sets this spacing so that the columns fill the width of the table. Horizontal rules are typeset using the `\hrule` command. The doubled (or Scotch) rules that appear at the top and bottom of a table can be achieved by enclosing the `tabular` environment within a `ruledtabular` environment. Rows whose columns span multiple columns can be typeset using L^AT_EX’s `\multicolumn{#1}{#2}{#3}` command (for example, see the first row of Table III).

TABLE III. This table demonstrates the use of `\multicolumn` in rows with entries that span more than one column.

Ion	1st alternative	D_{4h}^1	2nd alternative	1st alternative	D_{4h}^5	2nd alternative
K	$(2e) + (2f)$		$(4i)$	$(2c) + (2d)$		$(4f)$
Mn	$(2g)^a$		$(a) + (b) + (c) + (d)$	$(4e)$		$(2a) + (2b)$
Cl	$(a) + (b) + (c) + (d)$		$(2g)^b$	$(4e)^a$		
He	$(8r)^a$		$(4j)^a$	$(4g)^a$		
Ag			$(4k)^a$			$(4h)^a$

^a The z parameter of these positions is $z \sim \frac{1}{4}$.

^b This is a footnote in a table. It is supposed to set on the full width of the page, just as the caption does.

TABLE IV. A table with more columns still fits properly in a column. Note that several entries share the same footnote. Inspect the \LaTeX input for this table to see exactly how it is done.

	r_c (Å)	r_0 (Å)	Kr_0		r_c (Å)	r_0 (Å)	Kr_0
Cu	0.800	14.10	2.550	Sn ^a	0.680	1.870	3.700
Ag	0.990	15.90	2.710	Pb ^b	0.450	1.930	3.760
Au	1.150	15.90	2.710	Ca ^c	0.750	2.170	3.560
Mg	0.490	17.60	3.200	Sr ^d	0.900	2.370	3.720
Zn	0.300	15.20	2.970	Li ^b	0.380	1.730	2.830
Cd	0.530	17.10	3.160	Na ^e	0.760	2.110	3.120
Hg	0.550	17.80	3.220	K ^e	1.120	2.620	3.480
Al	0.230	15.80	3.240	Rb ^c	1.330	2.800	3.590
Ga	0.310	16.70	3.330	Cs ^d	1.420	3.030	3.740
In	0.460	18.40	3.500	Ba ^e	0.960	2.460	3.780
	^a Here's the first, 0.480 ref. 1.	18.90	3.550				

^bHere's the second.

^cHere's the third.

^dHere's the fourth.

^eAnd etc.

TABLE V. First narrow table.

Element Symbol	Element Name
H	Hydrogen

TABLE VI. Second narrow table, set alongside.

Trial	Time (s)
1	2.42
2	2.46
3	2.41

TABLE VII. Third narrow table, set alongside once again.

Case	Result
A	Pass
B	Fail
C	Pass

The tables in this document illustrate various effects. Lengthy tables may need to break across pages. A simple way to allow this is to specify the [H] float placement on the `table` environment. Alternatively, using the standard \LaTeX 2 ϵ package `longtable` gives more control over how tables break and allows headers and footers to be specified for each page of the table. An example of the use of `longtable` can be found in the file `summary.tex` that is included with the REVTeX 4 distribution.

There are two methods for setting footnotes within a table (these footnotes will be displayed directly below the table rather than at the bottom of the page or in the bibliography). The easiest and preferred method is just to use the `\footnote{#1}` command. This will automatically enumerate the footnotes with lowercase roman letters. However, it is sometimes necessary to have multiple entries in the table share the same footnote. In this case, create the footnotes using `\footnotemark[#1]` and `\footnotetext[#1]{#2}`. #1 is a numeric value. Each time the same value for #1 is used, the same mark is produced in the table. The `\footnotetext[#1]{#2}` commands are placed after the `tabular` environment. Examine the \LaTeX source and output for Tables I and IV for an illustration.

Sometimes it can be convenient to place multiple narrow figures or tables side-by-side to conserve space and meet any page length requirements for your conference. This can be done using `minipage` environments within the `table` or `figure` environment. Check the \LaTeX source and output for Tables V, VI, and VII for an example of how to do this. The vertical alignment of the `minipages` can be adjusted by changing the optional argument to the environment.

All AIP journals require that the initial citation of figures or tables be in numerical order. \LaTeX 's automatic numbering of floats is your friend here: just put each `figure` environment immediately following its first reference (`\ref`), as we have done in this example file.

CONCLUSION

In this section we welcome you to include a summary of the end results of your research.

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