Development of Magnetic Resonance Imaging Contrast Material for In vivo Mapping of Tissue Transglutaminase Activity

Galit Mazooz,1 Tevie Mehlman,2 Thung-Shen Lai,3 Charles S. Greenberg,3 Mark W. Dewhirst,4 and Michal Neeman1

1Departments of Biological Regulation and 2Biological Services, The Weizmann Institute of Science, Rehovot 76100, Israel; Departments of
3Medicine and 4Radiology Oncology, Duke University Medical Center, Durham, North Carolina

Abstract
Transglutaminases are a family of enzymes that play an important role in tissue remodeling by catalyzing covalent cross-links between proteins of the extracellular matrix. Elevated activity of transglutaminase was shown at the boundaries of invading tumors, in association with angiogenesis, in stabilization of atherosclerotic plaques, and in generation of blood clots. The aim of this work was to develop a low molecular weight substrate of transglutaminase that could serve for noninvasive magnetic resonance and optical mapping of transglutaminase-mediated cross-linking activity. A 2 kDa contrast material was generated which showed cross-linking by either tissue transglutaminase or factor XIII in the context of multicellular tumor spheroids or fibrin clots, respectively. Successful detection by nuclear magnetic resonance microscopy of transglutaminase-mediated cross-linking of the contrast material to MCF7 multicellular spheroids provides hope that this approach could potentially be developed for clinical demarcation of sites of transglutaminase activity. (Cancer Res 2005; 65(4): 1369-75)

Materials and Methods
Synthesis and Characterization of TGS-GdDTPA. High purity (>90% pure; high-performance liquid chromatography-purified, analyzed by mass spectrometry) biotin- or dansyl-labeled peptide (biotin/dansyl-H2NLYNLNQEQVSPK-KOC3H; b/d-TGS) was purchased from BACHEM, Inc. (Torrance, CA) and used for synthesis of H-biotin/dansyl-TGS-GdDTPA (b/d-TGS-GdDTPA). The results reported in this study arose from four independent syntheses with slight variations as listed in Table 1.

Briefly, for each synthesis, the peptide was dissolved in a minimal volume of HEPES 0.1 mmol/L (pH 8.8). The dissolved peptide was reacted with cyclic DTPA anhydride (Sigma, St. Louis, MO; 8.1 molar ratio relative to the peptide) suspended in filtered DMSO at room temperature, and the pH was adjusted to 8.5 using 5 N NaOH. The final concentration in the reaction solution was 0.0037 mol/L for the peptide and 0.03 mol/L for DTPA anhydride. The reaction product was dialyzed against water using tube-O-Dialyzer (Upstate, Lake Placid, NY; molecular weight cutoff limit of 1,000 Da), either before addition of gadolinium(III) chloride (GdCl3, Sigma) in sodium acetate 0.1 mol/L (pH 6.0 in the reaction mixture) in 1:1 molar ratio (syntheses 3 and 4) or, alternatively, after addition of excess gadolinium (Table 1; syntheses 1 and 2). The solution was stirred for 24 hours at 4°C after addition of GdCl3.

At the end of synthesis, the material was lyophilized for storage and was reconstructed in water for MRI measurements. The concentration of TGS...
Mass Spectrometry. Mass spectrometry was acquired on a Bruker reflex IIITM matrix assisted laser desorption/ionization time of flight mass spectrometer (Bruker, Bremen, Germany) equipped with delayed ion extraction, reflector, and 337 nm nitrogen lasers (Table 1; Supplemental Fig. S1). Each mass spectrum was generated from accumulated data of 200 laser shots.

Mass spectra of b/d-TGS-GdDTPA showed a well-defined peak corresponding with the reaction product for addition of GdDTPA. A high efficiency of labeling with GdDTPA was manifested by the lack of peak corresponding with the native peptide. Similar spectra were obtained also for d-TGS-GdDTPA (Supplemental Fig. S1).

**Table 1. Synthesis of b/d-TGS-GdDTPA**

<table>
<thead>
<tr>
<th></th>
<th>Synthesis 1</th>
<th>Synthesis 2</th>
<th>Syntheses 3 and 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dansyl-TGS (mg)</td>
<td>4</td>
<td>2</td>
<td>5.8</td>
</tr>
<tr>
<td>Biotin-TGS (mg)</td>
<td>8.8</td>
<td>8.8</td>
<td>8.8</td>
</tr>
<tr>
<td>HEPES reaction buffer (pH)</td>
<td>4</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>DTPA (mg)</td>
<td>4</td>
<td>2</td>
<td>&gt;8</td>
</tr>
<tr>
<td>pH</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Total reaction volume (mL)</td>
<td>0.4</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Dialysis</td>
<td>–</td>
<td>–</td>
<td>Against double distilled water</td>
</tr>
<tr>
<td>O-dialyzer tube, MW cutoff</td>
<td>1,000 Da</td>
<td>1,000 Da</td>
<td>0.89</td>
</tr>
<tr>
<td>GdCl₃ (mg)</td>
<td>4</td>
<td>2</td>
<td>0.89</td>
</tr>
<tr>
<td>Dialysis</td>
<td>Against</td>
<td>Against</td>
<td>–</td>
</tr>
<tr>
<td>O-dialyzer tube, MW cutoff</td>
<td>citrate buffer</td>
<td>citrate buffer</td>
<td></td>
</tr>
<tr>
<td>GdCl₃ (mg)</td>
<td>4</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Lyophilization</td>
<td>–</td>
<td>–</td>
<td>Reconstitution with 0.4 mL double distilled water</td>
</tr>
<tr>
<td>[d-TGS-GdDTPA] (mmol/L)</td>
<td>9.62</td>
<td>6</td>
<td>3.65</td>
</tr>
<tr>
<td>[b-TGS-GdDTPA] (mmol/L)</td>
<td>ND</td>
<td>ND</td>
<td>70</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>ND</td>
<td>ND</td>
<td>70</td>
</tr>
<tr>
<td>Mass spectrometry, peak of main product [M + H]⁺</td>
<td>ND</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Inductively coupled plasma-mass spectrometry [Gd] (mg/mL)</td>
<td>ND</td>
<td>15-20</td>
<td>14.7</td>
</tr>
<tr>
<td>Relaxivity (L/mmol/s)</td>
<td>4B and 5 1 and 44</td>
<td>2A and C</td>
<td></td>
</tr>
</tbody>
</table>

was determined spectroscopically by absorbance of tyrosine (measured at 280 nm). The recovery yield of the synthesis product after purification was ~70% (Table 1). The reaction product of all syntheses was analyzed by mass spectrometry, showing a single DTPA conjugated to b/d-TGS, with no peak corresponding to conjugation of two DTPA residues and in most reactions no residual b/d-TGS. The amount of Gd(III) was determined by inductively coupled plasma-mass spectrometry and relaxivity was measured by MRI (Table 1).

**Magnetic Resonance Measurements.** Nuclear magnetic resonance (NMR) microscopy measurements were done on a 400-MHz (9.4 T) wide-bore DMX spectrometer (Bruker, Karlsruhe Germany), equipped with a microimaging attachment with a 5 mm Helmholz radio frequency coil.

Longitudinal ($T_1$) relaxation time was measured from spin echo images (flip angle, 90°) acquired at a number of repetition times: 2,000, 1,000, 800, 500, 200, 100 ms; echo time = 8.9 ms; 128 × 128 matrix size; 2 averages; spectral width = 50,000 Hz; FOV = 0.5 × 0.5 cm; slice thickness 1 mm. $R_1$ maps were analyzed on an Indigo-2 work station using Matlab (The Math Works Inc., Natick, MA) by fitting the data to a single exponential to yield $R_1$ maps or for derivation of $R_1$ values from selected regions of interest. $R_1$ maps for each sample were generated, yielding $R_1$ values for each pixel, and further analyzed by means of region of interest and were assembled to yield the mean $R_1$ of the spheroid. $R_1$ histograms of the spheroids at indicated time, pre- and post-incubation with TGS-GdDTPA, were generated from regions of interest of all spheroids in the sample.

The $T_1$ (spin-lattice) relaxation times were measured using a 400-MHz (9.4 T) spectrometer. A spin echo sequence was employed to measure the relaxation time. The relaxation efficacy of the compound was then determined by calculating the relaxivity, defined as the slope of the function concentration (mmol/L) versus relaxation rate (s⁻¹).

**Tissue Transglutaminase Activity Assay.** 5-Biotin (amido)pentylamine (BP; Pierce, Rockford, IL) was used for calibration of the experimental procedures as general nonspecific transglutaminase substrate whereas TGS was used as a specific substrate. Transglutaminase activity was determined as previously reported using solid-phase microtiter plates coated with $N\_V\_N'$-dimethylcysline (20 mg/mL; refs. 15–17). After the unbound $N\_V\_N'$-dimethylcysline was discarded, the wells were blocked with 3% bovine serum albumin. Transglutaminase (13 μg/mL) and substrates (BP or b-TGS-GdDTPA) were added at the desired concentrations. After 45 minutes of incubation at 37°C, the liquid was discarded and the reaction was stopped by washing with EDTA. Bound biotinylated TGS was detected using streptavidin–alkaline phosphatase with phosphatase substrate (Sigma). Kinetic measurement of absorption at 405 nm was determined at 15-second intervals for a period of 5 minutes (VICTOR II; Wallac, 1420 Multilabel counter). Relative activity of tissue transglutaminase is expressed as units of absorbance.

**Monolayer and Multicellular Spheroid Culture.** MCF7 breast carcinoma spheroids were cultured in RPMI supplemented with 10% FCS (Biological Industries, Beit Haemek, Israel) and antibiotics (50 units/mL penicillin and 50 units/mL streptomycin). Aggregation of cells into large spheroids of about 0.75 to 1 mm was initiated by plating cells from confluent culture on agar-coated plates. After 48 hours the spheroids were transferred to a 500 mL spinner flask (Belco, Vineland, NJ); the medium was changed every other day for ~1 month (18).

Spheroids were incubated for 48 hours with BP and then were fixed for histology. Similarly, spheroids were incubated with h-TGS before the reaction with GdDTPA and histologic sections were stained with avidin FITC (Sigma) and cell nuclei were counterstained with Hoechst 33342 (Molecular Probes, Eugene, OR). For NMR microscopy, spheroids were incubated for 48 hours with b-TGS-GdDTPA.

**Histology.** Multicellular MCF7 spheroids were placed overnight in 4% formaldehyde buffered fixative solution. Fixed spheroids were embedded in paraffin blocks and sectioned serially at 4 μm thickness. Two serial slides from each spheroid batch was stained by H&E. In order to detect BP or b-TGS incorporation to the spheroids, deparaffinized slides (xylene for 5 minutes followed by sequential ethanol hydration and double distilled water) were stained with avidin FITC. For tissue transglutaminase expression the sections were stained with anti-Transglutaminase II Ab-3 (clones CUB7402 + TG100; mouse monoclonal antibody; Neomarker, Fremont, CA). Goat anti-mouse, conjugated to alkaline phosphatase, was used as second antibody and was visualized with Fast Red (Sigma).

The slides were counterstained with Mayer hematoxylin solution and examined with an Optiphot2 microscope (Nikon, Kanagwa, Japan) and photographed by a CCD camera (DVC Co., Austin, TX).
Peptide Cross-Linking into Fibrin Gel. Fibrinogen (10 mg) was dissolved in Tris-Cl buffer (pH 7.5), added to iodoacetamide (0.5 mg/mL) with thrombin and incubated with dansyl-TGS for at least 4 hours at 37°C for generation of fibrin clots. Materials that did not incorporate into the clot were removed by compressing the clot. The fibrin gels were then degraded with plasmin, and the amount of incorporated peptide was analyzed by the fluorescence of dansyl (19).

Results

Analysis of the Relaxivity of b-TGS-GdDTPA. High-performance liquid chromatography-purified (>90% pure) b/d-TGS was used for synthesis of b/d-TGS-GdDTPA (Table 1; Supplemental Fig. S1). Mass spectrometry of the product showed addition of a single DTPA, most likely a mixture of conjugation to either one of the two lysine residues at the C terminus of the peptide. The reaction product was further characterized by inductively coupled plasma-mass spectrometry to determine the concentration of Gd(III), spectroscopically to determine the final peptide concentration, and by MRI to determine the relaxivity.

Addition of b-TGS-GdDTPA (Table 1, synthesis 2) significantly increased the $R_1$ relaxation rate of water (Fig. 1). Avidin beads were added to sequester and precipitate the b-TGS-GdDTPA to the bottom of the NMR tube (Fig. 1A and B). Thus, the biotin residue was exploited for verifying that the observed relaxivity was indeed associated with b-TGS and not due to residual free Gd$^{3+}$ ions or GdDTPA (Fig. 1B and C). The elevated $R_1$ relaxation rate due to b-TGS-GdDTPA could be abolished by precipitating the b-TGS-GdDTPA using avidin beads (Fig. 1B and C). In that sample, the avidin beads visible at the bottom of the NMR tube showed enhancement and high $R_1$ relaxation rate due to the bound contrast material (Fig. 1B and C).

Control sample included b-TGS-GdDTPA incubated with pre-blocked avidin beads, in which biotin binding sites of the beads were pre-saturated with excess free biotin. These beads were unable to precipitate the peptide and, accordingly, the solution above the beads retained high $R_1$ relaxation. Additional control samples of water (no b-TGS-GdDTPA) with avidin beads or avidin beads pre-saturated with excess free biotin showed low $R_1$ relaxation rates very similar to that of free water.

The peptide relaxivity in syntheses 1 and 2 was estimated to be about 15 to 20 L/mmol/s after correcting for the excess free gadolinium. This value is significantly higher than the relaxivity of GdDTPA (4.2 L/mmol/s). In order to determine relaxivity more accurately, b-TGS-DTPA was synthesized and GdCl$_3·6$H$_2$O at defined concentration (as determined by inductively coupled plasma-atomic emission spectrometry) was added at equal molar ratio (Table 1, syntheses 3 and 4) to avoid contribution of excess free Gd$^{3+}$ ions, which could affect the relaxivity (Fig. 2A). The calculated relaxivity was 14.7 L/mmol/s.

Activity of TGS and TGS-GdDTPA as Transglutaminase Substrates. Tissue transglutaminase activity was verified by the cross-linking of a nonspecific tissue transglutaminase substrate (BP) to N$_2$N$_2$-dimethylcasein. Cross-linking activity showed saturation at tissue transglutaminase concentration of 1 µg/mL (Fig. 2B). A similar assay was used for testing cross-linking activity of

![Figure 1. Analysis of the relaxivity of b-TGS-GdDTPA. (A) $T_1$ weighted image (repetition time = 2000 ms; echo time = 8.9 ms) of b-TGS-GdDTPA (peptide) + avidin beads. (B) an example of $R_1$ map derived from the sample shown in A. (C) $R_1$ values determined for the following samples: avidin beads; avidin beads saturated with biotin + water; b-TGS-GdDTPA (peptide); peptide + avidin beads and peptide + avidin beads pre-saturated with biotin. Region of interest was selected for a region of free solution above the beads. Note that the high relaxivity due to b-TGS-GdDTPA was been sequestered by avidin beads, but not by pre-saturated beads, implying that the relaxivity is due to the biotinylated TGS-GdDTPA and not due to residual free Gd or GdDTPA.](https://www.aacrjournals.org/doi/fig/10.1158/0008-5472.CAN-04-1251)

Transglutaminase Imaging by MRI
transglutaminase on b-TGS-GdDTPA. Tissue transglutaminase showed significant cross-linking of b-TGS-GdDTPA to casein-coated solid-phase microtiter plates (Fig. 2C; synthesis 3).

The activity of d-TGS as a substrate for factor XIII (plasma transglutaminase) was tested in fibrin gel clots (Fig. 2D). Fibrinogen was cleaved by thrombin to fibrin and polymerized to gel. The fluorescence measurements of d-TGS after degradation of the fibrin clot by plasminogen allowed estimation of the concentration of the peptide, which was incorporated to fibrin on the order of 1 μmol/L. Control clots were tested with peptide (d-TGC) in which glutamine was replaced by glycine, thus making the peptide inactive for transglutaminase-mediated cross-linking. High fluorescence due to transglutaminase (factor XIII)-mediated cross-linking was observed only for d-TGS in the presence of Ca²⁺, whereas all control clots showed low fluorescence. Thus, no cross-linking was measured for the control sequence (d-TGC) or d-TGS in the absence of Ca²⁺ (addition of EDTA; Fig. 2D).

Expression and Activity of Transglutaminase in MCF7 Multicellular Tumor Spheroids. Immunohistochemistry revealed expression of tissue transglutaminase in the viable rim of MCF7 multicellular spheroids (Fig. 3A and B). Extracellular activity of transglutaminase was verified in MCF7 spheroids incubated with the nonspecific transglutaminase substrate (BP) for 48 hours. Bound BP was detected using avidin-FITC, showing significant fluorescence in the viable spheroid rim (Fig. 3D) that was not observed for spheroids incubated only with avidin-FITC (Fig. 3C). A low level of nonspecific binding of avidin-FITC was observed in the necrotic center in the presence (Fig. 3D) and absence (Fig. 3C) of BP. This result was consistent with the immunohistochemistry assay, which showed the localization of the enzyme around the viable rim (Fig. 3A).

Optical Detection of Tissue Transglutaminase-Mediated TGS Cross-Linking in Multicellular Spheroids. Based on the expression and activity of transglutaminase in multicellular spheroids, MCF7 spheroids were used as an in vitro tumor model for assessment of the feasibility for detection of transglutaminase activity through cross-linking of b/d-TGS-GdDTPA. Detectable cross-linking of b-TGS-GdDTPA (synthesis 2) was found in the viable rim of multicellular spheroids by staining with avidin-FITC. Binding of the peptide was found in the extracellular space surrounding cells in the viable rim of multicellular spheroids (Fig. 4A). Direct optical visualization of cross-linking of d-TGS-GdDTPA (synthesis 1) to the viable rim of multicellular spheroids was difficult due to the very low sensitivity for detection of fluorescence of dansyl. Cross-linking of the peptide in a punctate pattern was observed (Fig. 4B). One possibility is that transglutaminase is released from cells undergoing cell death in the tumor viable rim.

Detection of Tissue Transglutaminase Activity in Multicellular Spheroids by NMR Microscopy. MCF7 spheroids were incubated with d-TGS-GdDTPA (synthesis 1) and studied by NMR microscopy in comparison with control spheroids from the same stirred suspension culture. Spheroids were placed in culture medium in a 5 mm NMR tube (tube inner diameter, 4.2 mm). Relaxation map of b-TGS-GdDTPA relative to control spheroids; the spheroids were labeled with arrows (Fig. 5A and B; unpaired t test, P = 0.0004). RI histograms of spheroids that were incubated without TGS-GdDTPA (open circles) showed a shift in values with labeling (Fig. 5C).

Discussion

Transglutaminase activity is an important element in tissue remodeling providing mechanical strength by generation of
covalent cross-links between extracellular proteins. Specifically, tissue transglutaminase activity was shown at the advancing front of invading tumors, in association with angiogenesis, in stabilization of atherosclerotic plaques, and in generation of clots (20, 21). The aim of this study was to show the feasibility of generation of a low molecular weight substrate of transglutaminase that could serve for noninvasive MRI and optical mapping of transglutaminase-mediated cross-linking activity.

The candidate transglutaminase substrate (TGS) tested here included a peptide providing specific recognition sequence of transglutaminase, along with dansyl or biotin tags for fluorescence imaging and GdDTPA for MRI contrast. Thus, b-TGS was visualized using avidin-FITC and streptavidin alkaline phosphatase, d-TGS was detected by fluorescence of dansyl, and GdDTPA-labeled TGS was detectable by MRI from changes in $R_1$ relaxation rates. Moreover, the biotin tag was further used for sequestration of the contrast material in MRI experiments, thus verifying that the observed magnetic resonance relaxation was due to the biotinylated peptide. Cross-linking of TGS could be detected on casein-coated plates, a classic assay of transglutaminase.

Figure 3. Expression of tissue transglutaminase in MCF7 multicellular spheroids detected with histology and confocal microscopy. (A) MCF7 spheroids stained with Transglutaminase II Ab-3 (clones CUB7402 + TG100) mouse monoclonal antibody. Goat anti-mouse, conjugated to alkaline phosphatase, was used as second antibody and was visualized with Fast Red. The insert shows magnified view of H&E histologic staining of cells in the viable rim. (B) same section as in A, Fast Red detected by fluorescence microscopy. (C) MCF7 spheroids were incubated with avidin FITC for 45 minutes in the absence of BP. (D) MCF7 spheroids were incubated with avidin-FITC for 45 minutes after 48 hours of incubation with BP.

Figure 4. Fluorescence detection of transglutaminase-mediated cross-linking of transglutaminase to multicellular spheroids. (A) fluorescence image of d-TGS-GdDTPA (340 nm/520 nm). Arrows, cross-linking product between d-TGS-GdDTPA and the surface of the spheroid. (B) Inverted fluorescence microscopy of TGS binding to MCF7 spheroids. MCF7 spheroids were incubated with b-TGS-peptide for 48 hours followed by staining with avidin-FITC and Hoechst (Molecular Probes). Hoechst stained the nuclei whereas fluorescence of avidin-FITC was concentrated at the viable rim of the spheroid and around the cells. The scale bars of the images are 50 μm.
activity, as well as in fibrin clots and in the rim of multicellular spheroids.

The ability to detect factor XIII-mediated cross-linking of TGS to fibrin clots and tissue transglutaminase-mediated cross-linking to multicellular tumor spheroids offers hope that it would be possible to further develop this approach for clinical imaging of transglutaminase activity in clotting and for demarcation of tumor boundaries. Moreover, the ability to detect contrast changes by NMR microscopy of multicellular spheroids showed that the cross-linking activity generated by endogenously produced transglutaminase in tumors was sufficient for detection of significant contrast changes by MRI.

Sensitivity for detection of paramagnetic contrast material by MRI depends on the relaxivity, which in turn depends on the interaction of the paramagnetic ion with water and thus on the exchange of water in the inner sphere, and on the rotation time of the contrast material. Preliminary analysis suggests that the high sensitivity shown here for detection of the contrast material can be attributed not only to high binding efficiency but also to relaxivity that was higher than most low molecular weight contrast agents (22, 23). Analysis of the physical basis for the mechanism of relaxivity of this contrast material may aid future design of high-relaxivity low molecular weight contrast agents.

Sensitivity for detection of contrast in vivo would depend not only on the specific relaxivity and activity as shown here. Additional factors that would impact applicability in vivo include delivery of the material to the site of enzymatic activity and its retention in the site of activity. For both, targeting transglutaminase activity seems to be ideal for the sake of molecular imaging. The enzymatic activity is extracellular and occurs predominantly in regions of tissue remodeling and angiogenesis where vessels are typically hyperpermeable, thus allowing easy access. Moreover, retention of the contrast material at the site of interest is guaranteed by covalent cross-linking, which should provide significant dynamic separation between specific and nonspecific distribution. The specificity of the contrast can also be improved by induced removal of unbound magnetic resonance contrast material using avidin chase (24, 25).

Clearly, many hurdles still remain before the clinical application. The clearance of the peptide might be too rapid to allow sufficient binding that would enable detection. Thus, the material might need to be further modified to increase its half life in circulation. In addition, the stability and toxicity of the material and its breakdown products should be determined. In particular, gadolinium chelation might be compromised by binding to the peptide. Thus, structural modifications might be required and should be tested in preclinical models. A similar approach was reported recently for imaging the activity of factor XIII in atherosclerotic plaques in animal models by near-IR imaging (13, 14).

In summary, we showed here the development of a novel magnetic resonance contrast material, which can be applied for noninvasive imaging of transglutaminase activity. This approach opens possibilities for the study of the multiple biological processes in which transglutaminases participate including angiogenesis, tumor growth, and stabilization of fibrin clots. The high sensitivity provided by efficient extracellular retention and high relaxivity suggests that this approach can be tested in vivo in animal models, and could perhaps be further developed towards clinical demarcation of sites of transglutaminase activity in atherosclerotic plaques and at the advancing fronts of invading tumors.

Acknowledgments


Grand support: Varian Biosynergy.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Zishan Haroon for his contribution in the original development of the ideas tested in this article; Dr. Alla Shainskaya and Dalit Merhav from the Mass Spectrometry Units at Weizmann; Dr. Fortune Kohen for the development of the synthesis protocols; Miriam Cohen and Prof. Benjamin Geiger for the fluorescence microscopy of danny; and Hagit Dafni for the Matlab analysis.
References


Development of Magnetic Resonance Imaging Contrast Material for \textit{In vivo} Mapping of Tissue Transglutaminase Activity

Galit Mazooz, Tevie Mehlman, Thung-Shen Lai, et al.