Investigating the Potential of *Escherichia coli* to Promote Kidney Stone Formation

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**Introduction**

Kidney stones may either be classified as metabolic stones or as infection-induced stones (Gomez-Nunez et al. 2009) and both types tend to be associated with urinary tract infections (UTIs). The strong correlation of UTIs with nephrolithiasis may simply demonstrate the increased susceptibility to infection of tissue damaged by a pre-existing stone, but, in some cases, the UTI may be able to serve as the direct or indirect cause of stone formation itself (Burrall et al. 2004, Gomez-Nunez et al. 2009, Lindberg 2001, Pearson et al. 2008).

As renal calculi form bacterial cells may become embedded within the stone matrix and recent studies have suggested that the location of these embedded bacteria within the matrix may be indicative of the timing of the infection relative to the onset of stone formation (Tavichakorntrakoo et al. 2012).

*Escherichia coli* is the single most common bacterium isolated from the stone matrices and urine of patients who have kidney stones (Tavichakorntrakoo et al. 2012). The predominance of *E. coli* in both urine and throughout stone matrices, including the stone nidus, suggests a possible correlation between this bacteria and nephrolithogenesis (Tavichakorntrakoo et al. 2012). The purpose of this research was to investigate the role of *E. coli* in calcium oxalate crystallization.

**Results**

With the agarose plates set up as described, the calcium chloride and sodium oxalate solutions diffuse outward and combine in the region between the two wells; a zone of precipitation is observed as calcium oxalate crystals are formed. The density of the calcium oxalate precipitate was observed to be directly proportional to the density of *E. coli* in the agarose plates (Figure 1) suggesting that *E. coli* cells directly or indirectly promote calcium oxalate crystal formation.

![Figure 1: Crystallization of calcium oxalate increased as the volume of *E. coli* suspension incorporated in agarose plate increased.](image)

**Discussion**

- *E. coli*-based UTIs may play a role in kidney stone formation in vivo (Tavichakorntrakoo et al. 2012)
- The agarose plate assay described in this report allows crystals to become trapped upon formation.
- Some feature of the bacterial cell may serve as a nucleus for crystal formation or that some byproduct of *E. coli* metabolism may alter the pH or otherwise affect the local environment to promote crystallization.

**Material and Methods**

- *E. coli* was grown and harvested by centrifugation at 10,000 rpm for 5 min at 4°C and washed 3 times with sterilized isotonic saline (0.9% NaCl) to eliminate medium.
- Washed *E. coli* cells were re-suspended in sterile 0.9% NaCl to a standardized cell density.
- Agarose pour plates (1% (w/v) agarose, 10 mM Tris, 90 mM NaCl, pH 7.4) were prepared to contain 5 x 10^5, 5 x 10^6, and 5 x 10^7 CFU/mL *E. coli* cells.

**Assays**

- Two diverging wells were created by cutting 5 cm trenches in the agar as shown in Figure 1.
- To one well 1 mL sodium oxalate solution (50 mM sodium oxalate, 10 mM Tris, 90 mM NaCl, pH 7.4) was added and 1 mL calcium chloride solution (50 mM calcium chloride, 10 mM Tris, 90 mM NaCl, pH 7.4) was added into the second.
- All plates were incubated at 37°C for up to 48 hours.
- Crystal formation in between the region of the two wells were visualized using ImageQuant (Trans-UV light, clear tray, 2 seconds exposure)

![Figure 2: Two diverging 5 cm trenches were cut into the agarose as indicated](image)

**Reference**